Serial No. 09/538,396
Group Art Unit: 1638

REMARKS

Reconsideration and entry of the present amendment after final is respectfully requested.

Status of the Claims:

Claims 2-8, 12, 14, and 18-38 were pending after submission of 13 June 2003. Claims 9-11 have been cancelled in the current amendment, they were previously withdrawn from examination. Applicants reserve the right to pursue the contents of these claims in continuing applications. Claims 2-8, 12, 14, and 18-38 remain under examination after amendment. Claims 12, 14, and 20 have been amended regarding the encoded polypeptide, the phrase "involved in DNA double strand break repair" has been replaced with the phrase "binds to a MRE11 polypeptide". Support for the amendments is found in the claims as originally filed, and throughout the specification, particularly page 1, lines 30-32. No new matter has been added.

Rejections under 35 U.S.C. §112, 1st Paragraph - Enablement:

Claims 2-8, 12, 14, and 18-38 are rejected under 35 U.S.C. §112, first paragraph as failing to comply with the enablement requirement. The rejection is repeated for the reasons of record as set forth in the Office Action of 1/13/03.

The Action asserts, briefly, that given the complexity of DNA repair, including the involvement of other proteins, it is unclear what agronomic benefit a transgenic plant with modulated Rad50 levels would have, and unclear how modulating the level of Rad50 would affect DNA repair or polynucleotide integration. Therefore the Action concludes that one skilled in the art would not know how to use SEQ ID NO: 1, and sequences having 90% and 95% to the disclosed sequences, without undue experimentation.

Applicants respectfully disagree, for the reasons of record (*e.g.*, see response filed 6/13/03), some of which may be reiterated for clarity. Applicants note that

claims 12, 14, and 20 have been amended, as described above, to recite polypeptides that "bind to a MRE11 polypeptide".

Applicants have provided full-length sequences, demonstrated conserved functional motifs (*e.g.*, page 2, lines 17-24, and Example 4), extensive overall homology, direction regarding nucleotide and amino acid substitutions, and percent identity comparisons (*e.g.*, Appendix A and D; page 5, line 20 – page 6; and line 25; page 16, line 1 – page 20, line 20 of the specification), Rad50 function in DNA break repair and interaction with Mre11 (*e.g.*, page 1, line 15 – page 2, line 14), vector construction, plant transformation, and methods to modulate the level of Rad50 (e.g., page 37, line 5 - page 42, line 22; page 44, line 28 – page 47, line 25; page 47, line 28 – page 49, line 26; page 50, line 29 – page 52, line 31; and page 53, line 1 – page 54, line 6). The Examiner is reminded that the need for routine experimentation and screening is not considered undue (see MPEP 2164.01):

> The test of enablement is not whether any experimentation is necessary, but whether, if experimentation is necessary, it is undue. *In re Angstadt*, 190 USPQ 214 (CCPA 1976). An extended period of experimentation may not be undue if the skilled artisan is given sufficient direction or guidance. *In re Colianni*, 195 USPQ 150 (CCPA 1977) (Miller, J., concurring). The experimentation required, in addition to not being undue, must not require ingenuity beyond that expected of one of ordinary skill in the art. *In re Angstadt*, supra. For example, in one instance a "few hours" of experimentation to determine process parameters was not considered to be undue in view of the nature of the invention (preparation of oxygenated hydrocarbons). *In re Borkowski*, 164 USPQ 642 (CCPA 1970). In Tabuchi v. Nubel, 194 USPQ 521 (CCPA 1977) a screening procedure which took 15 calendar days was not considered undue experimentation because the test was both simple and straightforward and because of its demonstrated success in producing the desired result.

Further, the Examiner has not given specific reasons, or provided specific citations to support the conclusions that modulation of the level of Rad50 would not have an effect on the plant.

Modulation of Rad50, thereby modulating the level of DNA repair in the plant, is predicted *to increase the efficiency of incorporation of heterologous nucleic acids*

in the genome of a plant. For the record, Applicants disagree with the Examiner's conclusion that modulation of Rad50 must result in an agronomic benefit to the transgenic plant. Applicants utility, *to use Rad50 to modulate the efficiency of incorporation of a transgene into the plant genome* (page 2, lines 10-14) does not require that Rad50 confer any particular agronomic phenotype to the plant.

Applicants note that they have provided another component of the DNA repair complex, Mre11, in U.S. Patent 6,646,182, issued Nov. 11, 2003, thereby demonstrating the presence of Mre11 in plants. Also, Gallego *et al.* have demonstrated Rad50 involvement in DNA repair and meiosis in *Arabidopsis* (*Plant J.* 25:31-41 2001, submitted in Appendix C). Applicants have modified the Multiple Sequence Alignment (Appendix A) to include the conserved motifs shown in Example 4 of the specification, currently presented as Appendix D. Applicants note that the alignment is similar to the one shown by Gallego *et al.* (supra), which shows higher conservation of the N- and C-terminal regions of Rad50. Appendix D also includes the results of an analysis performed using the Lion BioScout software package, and Hmmerpfam search using GCG software, both of which detect the known Rad50 Zn-hook Pfam domain (e = 6.9e-07).

Applicants assert this support fully enables one of skill in the art to make and use the full-breadth of the invention without undue experimentation. Absent of any evidence from the Examiner supporting the conclusion that modulation of Rad50 would not effect the plant, Applicants believe this rejection cannot be supported. Therefore Applicants respectfully request reconsideration and that the rejection of claims 2-8, 12, 14, and 18-38 under 35 U.S.C. §112, first paragraph for lack of enablement be withdrawn.

Rejections under 35 U.S.C. §112, 1st Paragraph, Written Description:

Claims 2-8, 12, 14, 20 and 23-38 are rejected under 35 U.S.C. §112, first paragraph, as containing subject matter not sufficiently described in the specification

to indicate the inventor(s) had possession of the invention. The rejection is repeated for the reasons of record as set forth in the Office Action of 1/13/03.

Briefly, the Action asserts that the Applicant has not described a representative sample of the genus, and that the conserved domains are not unique to Rad50 proteins, but are common to all DNA repair proteins. The Action also asserts that Example 14 of the Revised Interim Written Description Guidelines is not applicable because claims drawn to polynucleotides having 95% sequence identity are not rejected in this Action.

Applicants respectfully disagree, for the reasons of record (*e.g.*, see response filed 6/13/03), some of which may be reiterated for clarity. As is stated in the MPEP 2163 (see p. 2100-168) the written description for a claimed genus may be satisfied by "disclosure of relevant, identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show that the applicant was in possession of the claimed genus." Applicants respectfully submit that the present application meets this standard by the disclosure of the structures of the full-length Rad50 polynucleotide (SEQ ID NO: 1), which encodes a full-length Rad50 polypeptide (SEQ ID NO: 2) which have the structural/chemical properties of significant sequence identity to known Rad50 polynucleotides and polypeptides, conserved domains (see, for example, Example 4 and the Multiple Sequence Alignment), and the functional characteristic of Rad50 polypeptide binding to MRE11 polypeptides.

Regarding the applicability of Example 14, for the record Applicant notes that while claims 18 and 21, directed to sequences having 95% identity to either SEQ ID NO: 1 or SEQ ID NO: 2, are not included in this rejection, they are also not allowed. Also, the Examiner has not made clear why Example 14 does not apply, other than focusing on the specific percent identity recited and not the substantive features of the structural and functional elements. Applicants maintain that the current claims, including those directed to 90% sequence identity, meet the Written Description

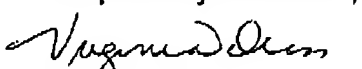
standard as set forth in the 35 U.S.C. §112, described MPEP, and shown in Example 14 of the Revised Interim Written Description Guidelines.

Applicants have coupled structural, chemical, and functional properties to describe polynucleotides having 90% and 95% sequence identity to SEQ ID NO: 1, or encoding polypeptides that have 90% and 95% sequence identity to SEQ ID NO: 2 such that a person skilled in the art can envisage the claimed invention, thereby meeting the 35 U.S.C. §112, first paragraph written description requirement. Therefore Applicants respectfully request reconsideration and that the rejection of claims 2-8, 12, 14, 20 and 23-38 under 35 U.S.C. §112, first paragraph for lack of written description be withdrawn.

CONCLUSION

In light of the foregoing remarks and amendments, it is believed that claims 2-8, 12, 14 and 18-38 are in condition for allowance. Withdrawal of the outstanding rejections and allowance of all of the remaining claims is respectfully requested. The Examiner is invited to telephone the Applicants representative to expedite prosecution and allowance.

Respectfully submitted,



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APPENDIX C

The Plant Journal (2001) 25(1), 31–41

Disruption of the *Arabidopsis RAD50* gene leads to plant sterility and MMS sensitivity

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Summary

The Rad50 protein is involved in the cellular response to DNA-double strand breaks (DSBs), including the detection of damage, activation of cell-cycle checkpoints, and DSB repair via recombination. It is essential for meiosis in yeast, is involved in telomere maintenance, and is essential for cellular viability in mice. Here we present the isolation, sequence and characterization of the *Arabidopsis thaliana RAD50* homologue (*AtRAD50*) and an *Arabidopsis* mutant of this gene. A single copy of this gene is present in the *Arabidopsis* genome, located on chromosome II. Northern analysis shows a single 4.3 Kb mRNA species in all plant tissues tested, which is strongly enriched in flowers and other tissues with many dividing cells. The predicted protein presents strong conservation with the other known Rad50 homologues of the amino- and carboxy-terminal regions. Mutant plants present a sterility phenotype which co-segregates with the T-DNA insertion. Molecular analysis of the mutant plants shows that the sterility phenotype is present only in the plants homozygous for the T-DNA insertion. An *in vitro* mutant cell line, derived from the mutant plant, shows a clear hypersensitivity to the DNA-damaging agent methylmethane sulphonate, suggesting a role of *RAD50* in double-strand break repair in plant cells. This is the first report of a plant mutated in a protein of the Rad50–Mre11–Xrs2 complex, as well as the first data suggesting the involvement of the Rad50 homologue protein in meiosis and DNA repair in plants.

Keywords: ***Arabidopsis, RAD50,* DSB repair, sterility, recombination.**

Introduction

The repair of DNA double-strand breaks (DSBs) involves genetic recombination. Fundamentally, two different forms of recombination are involved: those involving DNA sequence homology between the participating DNA molecules, and those that appear to act independently of such homology, called homologous recombination (HR) and non-homologous end-joining (NHEJ), respectively. Considerable advances in the understanding of these mechanisms have been made in recent years, both in terms of the proteins involved and of their relative contribution to repair of DSBs in different organisms and/or cell types. These issues are the subject of a number of recent reviews (Fox and Smith, 1998; Jeggo, 1998; Paques and Haber, 1999; Petrini *et al.*, 1997; Roeder, 1997; Smith, 1998; Smith and Nicolas, 1998; Tsukamoto and Ikeda, 1998).

Much of our understanding of the proteins implicated in the processes of recombination and DSB repair comes from studies of the yeast *Saccharomyces cerevisiae*. Homologous recombination in yeast cells is largely under the control of the genes in the *RAD52* epistasis group, which include *RAD50–59*, *XRS2* and *MRE11* (Ajimura *et al.*, 1993; Game, 1993; Haynes and Kunz, 1981). These genes were mostly identified as being needed for the repair of ionizing radiation-induced DNA damage, and are also needed for meiosis. Deletion of these genes in yeast generates defects in both recombination and DNA repair with differing phenotypes in terms of recombination and the repair of DNA double-strand breaks. Mutants of *rad51, rad52, rad54, rad55, rad57* and *rad59* show defects in HR, while *rad50* and *mre11* mutants have shown defects in NHEJ and are hyper-rec for HR.

Double-strand breaks are repaired by both HR and NHEJ in all cell types examined; however, the preferred mechanism of recombinational DNA repair differs significantly in a given organism. In particular, it is clear that non-homologous recombination is much more frequent than homologous recombination in mammalian and plant cells, whereas yeast cells rely almost entirely on homology-based recombinational DNA repair. The basis of the strong preference for illegitimate recombination in mammalian and plant cells is not fully understood. Both pathways have a common initial substrate (DSB), the processing of which may be channelled into either HR or NHEJ. This channelling is apparently determined by the initial metabolism of the DSB itself, and it has recently been suggested that it may result from a competition for binding the DNA ends between the Ku and Rad52 proteins (VanDyck *et al.*, 1999). In this context, the Rad50/Mre11/Xrs2 complex is of particular interest, as mutants show a weak hyper-HR and strong hypo-NHEJ phenotype in mitotic yeast cells (Boulton and Jackson, 1998; Moore and Haber, 1996; Schiestl *et al.*, 1994; Tsukamoto *et al.*, 1996; Tsukamoto *et al.*, 1997). However, in some assays *RAD50* is needed for intrachromosomal recombination (Elias-Arnanz *et al.*, 1996; Rattray and Symington, 1995; Tran *et al.*, 1995). *Rad50*⁻ mutants show altered processing of DNA ends during recombination (Ivanov *et al.*, 1994; Sugawara and Haber, 1992). This complex has also been implicated in the regulation of the response of yeast cells to DNA damage (Kiromani and Muniyappa, 1997; Lee *et al.*, 1998).

Homologues of many of these yeast genes have been cloned from different organisms (see reviews by Kanaar and Hoeijmakers, 1997; Paques and Haber, 1999), including *RAD50* genes from *S. cerevisiae* (Alani *et al.*, 1989); *S. pombe* (Saunders, 1999); man (Dolganov *et al.*, 1996); mouse (Kim *et al.*, 1996); and *Caenorhabditis elegans* (Offenberg and Heyting, 1996). Rad50 shows homology to the *Escherichia coli* SbcC protein (Sharples and Leach, 1995), and has been shown to form a complex with the Mre11, Xrs2 and Lig4 proteins. The Rad50/Mre11 complex is involved in DSB repair via NHEJ in mammalian cells, and the null mouse *rad50* mutant is inviable both in cultured ES cells and in developing mouse embryos (Luo *et al.*, 1999). This cell-lethal phenotype has also been seen in mouse *mre11* mutants (Xiao and Weaver, 1997), as well as chicken (Sonoda *et al.*, 1998) and mouse *rad51* mutants (Lim and Hasty, 1996; Sharan *et al.*, 1997; Tsuzuki *et al.*, 1996). Interestingly, *rad52* mutant mice are viable and show increased radioresistance (Rijkers *et al.*, 1998; Yamaguchi-Iwai *et al.*, 1998).

A number of radiation-sensitive plant mutants have been isolated in recent years (reviewed by Britt, 1999; Gorbunova and Levy, 1999; Mengiste and Paszkowski, 1999). The mutated genes of methylmethane sulphonate (MMS) and UV-hypersensitive *Arabidopsis* mutants have recently been identified: a ribosomal S27 protein (Revenkova *et al.*, 1999); the *Arabidopsis* Rad1 homologue protein (Gallego *et al.*, 2000; Liu *et al.*, 2000); and a member of the structural maintenance of chromosomes protein family (SMC; Mengiste *et al.*, 1999; Strunnikov, 1998; Strunnikov *et al.*, 1993). Rad50 is also a member of this family. Plant homologues of *RAD51* (Doutriaux *et al.*, 1998; Smith *et al.*, 1996) and *MRE11* (Hartung and Puchta, 1999) have been isolated, and the rapid progress of the *Arabidopsis* sequencing project makes it likely that many other genes implicated in these processes will be isolated in the near future.

As part of our investigation of the control of the early events of DSB repair and recombination in plants, we have isolated the *Arabidopsis thaliana* homologue of the *RAD50* gene, and here we describe the isolation, sequencing and preliminary characterization of this gene. Furthermore, we have identified a *rad50* mutant plant that presents a sterility phenotype in agreement with the role of this protein in meiosis in yeast cells. We also show that homozygous mutant cell lines present hypersensitivity to MMS, suggesting (as has been shown in yeast cells) a role for *RAD50* in DSB repair in plants. This is the first evidence of the implication of the *RAD50* gene in meiosis as well as in DSB repair in plants.

Results and Discussion

The Arabidopsis thaliana RAD50 *homologue*

A 700 bp *A. thaliana* cDNA clone with homology to the carboxy-terminal region of the yeast *Rad50* gene was detected in a screen of the *Arabidopsis* cDNA expression library with an antibody against a peptide sequence from the cytoplasmic domain of vertebrate beta 1 integrin. Based on this sequence (a kind gift from P. Nagpal and R. Quatrano), oligonucleotides were designed and a fragment of 1.5 kb of genomic sequence was amplified from genomic DNA prepared from an *Arabidopsis* cell-suspension culture. This DNA fragment was used to screen a genomic lambda bank prepared with DNA from the same cells, permitting the identification of two lambda clones with overlapping inserts spanning approximately 22 kb of genomic DNA and including the *Arabidopsis RAD50* homologue locus. Sequencing of 10 kb from the inserts of these clones showed that they spanned the entire *AtRAD50* gene (data not shown). Our genomic DNA sequence has recently been confirmed by (and is identical to that of) the *Arabidopsis* genome sequencing project (Lin *et al.*, 1999).

The cDNA encoding the *Arabidopsis RAD50* homologue was isolated by RACE-PCR with the Marathon Kit (Clontech Inc., Palo Alto, CA, USA). Poly(A)+ RNA was prepared from total RNA isolated from the *A. thaliana* cell

suspension; double-stranded cDNA was synthesized and adapters were ligated to the two ends. This library of adapter-ligated cDNA was PCR amplified using a *RAD50* specific primer and primers against the adapter sequences. A PCR fragment of 4.3 kb was isolated on agarose gel and cloned into pGEM-Teasy (Promega Inc., Madison, WI, USA). Sequencing of the 4305 bp insert clone confirmed that it includes the entire *AtRAD50* cDNA, and that this cDNA corresponds to the genomic clones (above). The gene covers 8486 bp and contains 27 exons; all 5′ donor sites contain the AG/GT conserved junction; and the conserved AG is present in all the 3′ acceptor sites. We have submitted this mRNA sequence to GenBank (Gallego *et al.*, 1999). The computer prediction of the mRNA sequence of this gene from the genome sequencing project (Lin *et al.*, 1999) misplaces a number of the intron–exon borders, and thus predicts a protein with 53 inserted and 32 deleted amino acids relative to that predicted from our cDNA sequence.

Amino acid comparison of Rad50 *homologues.*

The cloned cDNA from *Arabidopsis* presents a putative methionine initiation codon at nucleotide residue 146 in the first exon. No other open reading frames were detected. The termination codon of the open reading frame is located at nucleotides in position 4097, giving a predicted protein of 1316 amino acids. A 3′-untranslated region covers 181 nt before the short poly(A) tail present in the cDNA clone. The length of the protein is strongly conserved in all organisms in which it has been studied: the human, mouse and yeast Rad50 proteins have 1312 amino acids, while the *C. elegans* Rad50 has 1298 and the *Arabidopsis RAD50* cDNA reported here predicts a protein of 1316 amino acids. Amino acid sequence comparison with the known Rad50 proteins shows a high conservation at the N- and C-terminal regions. In particular, the amino-terminal 190 amino acid region of the *Arabidopsis* protein has 56 and 52% sequence identity with the yeast and human Rad50 proteins, respectively. The carboxy-terminal 207 amino acids show >62 and 52% identity with the corresponding region present in the yeast and human Rad50 proteins, respectively (Figure 1a). The overall amino acid identity of the predicted *Arabidopsis* protein is of 29.8% with the human and 27.3% with the yeast proteins, respectively. The *Arabidopsis* Rad50 protein predicted from the cDNA sequence contains 18.2% of acidic amino acids and 16.9% of basic residues. The protein is predominantly hydrophilic, with 30.3% of hydrophobic residues. It presents three glycosylation motifs in positions 396–399, 419–422 and 620–623. A type-A ATP-binding side is present in the amino-terminal region at amino acid positions 34–41. A Walker B motif is present at the carboxy-terminal region between amino acids 1235 and 1242. Checking of the predicted AtRad50 amino acid sequence for plant localization signals with the PSORT program (http://psort.nibb.ac.jp/), yields a putative nuclear localization signal with certainty of 0.350 (success). No chloroplast or mitochondrial localization sequences were identified in the sequence by PSORT. Figure 1(b) shows the output from the COILS program (http://ulrec3.unil.ch/software/coils_FORM.html; Lupas *et al.*, 1991). It can be seen that the predicted AtRad50 protein conserves the central coiled-coiled conformation domain seen in the yeast and human proteins (Dolganov *et al.*, 1996).

Chromosomal localization of Arabidopsis RAD50 *locus*

Southern analysis of DNA prepared from *Arabidopsis* suspension cells show a unique band after digestion with six different restriction enzymes (Figure 2a), suggesting that the Arabidopsis *RAD50* gene is present as a single-copy gene within the *A. thaliana* genome.

Using PCR amplification of DNA prepared from the *Arabidopsis* YAC bank (Creusot *et al.*, 1995), we have mapped the *RAD50* gene on chromosome II (YAC CIC 11E1L) near the marker TEn5 (data not shown). Recently, genomic sequencing results of *Arabidopsis* chromosome II BAC F22D22 has confirmed this mapping (Lin *et al.*, 1999).

Expression of the RAD50 *gene in* Arabidopsis

Northern analysis of RNAs prepared from *Arabidopsis* suspension cells as well as different plant tissues shows a single mRNA species, the length of which corresponds to that of the cDNA (Figure 2b). This has been confirmed by Northern analysis of poly(A)+ RNA from suspension cells and flower buds, as well as by RT–PCR analysis (data not shown).

Studies of human and mouse *RAD50* gene expression have shown the presence of alternative spliced transcripts (Kim *et al.*, 1996; Kim *et al.*, 1999). We were unable to detect alternative transcripts in *A. thaliana* using both Northern and PCR analysis. The *Arabidopsis RAD50* mRNA is expressed in all cell tissues analysed- however, stronger levels were found in fast growing cells such as cell-suspension culture, young primary roots and flowering structures. Tissue from flower buds and mature flowers contains a relatively high proportion of cells undergoing division, processes that appear to be correlated with a high level of expression of *RAD50*. Maximum expression of human and mouse *RAD50* genes has been detected in the testis (Dolganov *et al.*, 1996; Kim *et al.*, 1996). Thus, although the transcript is enriched in meiotic tissues, expression of the *AtRAD50* gene is not specific to meiotic cells. This pattern of expression is reminiscent of that previously reported for the *Arabidopsis RAD51* homologue

(a)

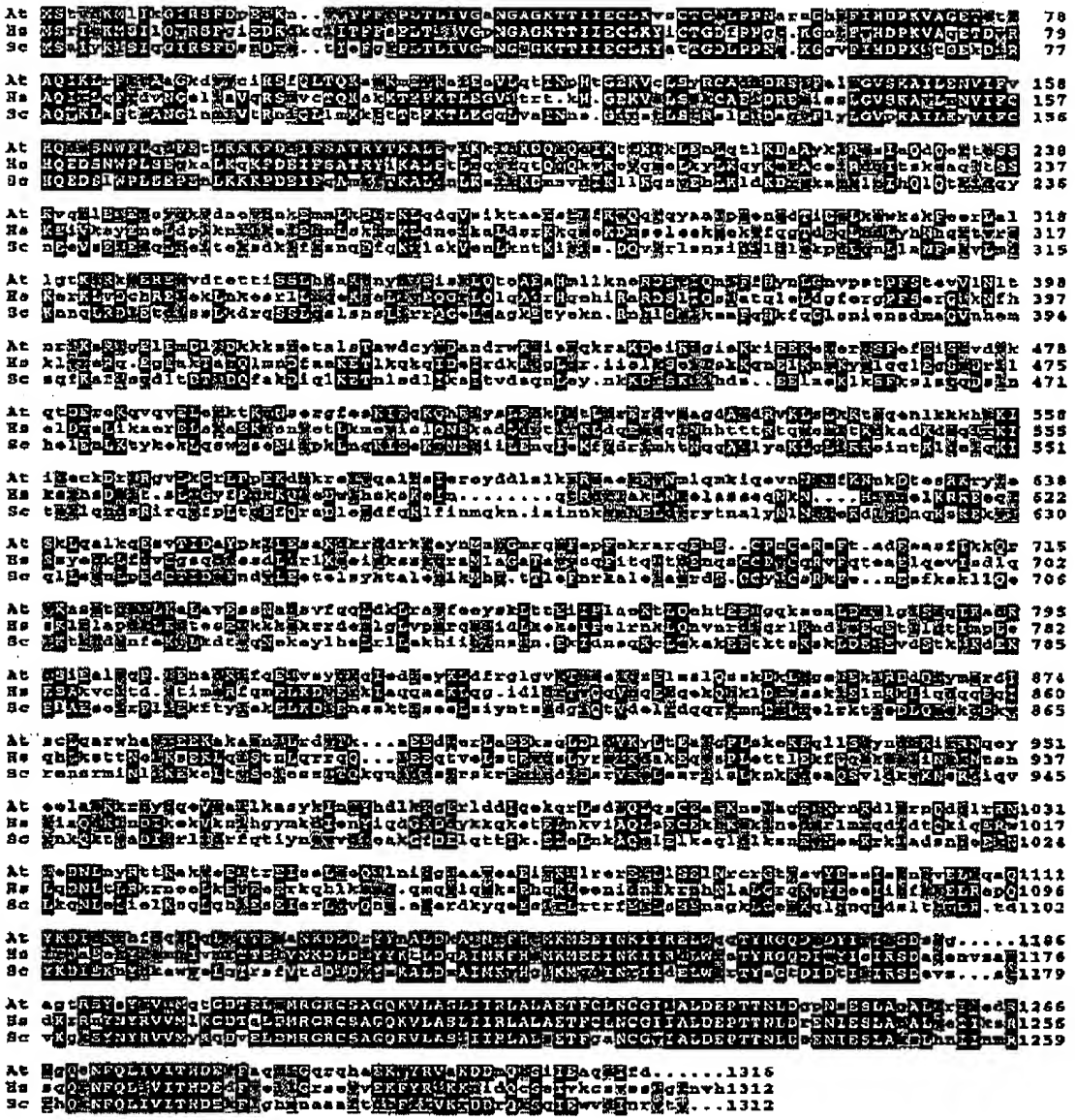

Figure 1. Comparison of AtRad50 and other Rad50 proteins.

(a) Alignment of *Rad50* homologues from *Arabidopsis* (At), man (Hs) and *S. cerevisiae* (Sc). Identical residues between two or more sequences are printed as white, upper-case letters outlined in black; conservative substitutions as black, lower-case letters outlined in grey; non-conserved residues are in lower case on white. Numbers to the right of each line indicate the position in the amino acid sequence of the last residue on that line. The sequences were aligned with the CLUSTALX program using the BLOSUM weighting matrix (Thompson *et al.*, 1994).

(b) Comparison of predicted coiled-coil structure of the *Arabidopsis* (1316aa), human (1312aa) and *S. cerevisiae* (1312aa) Rad50 proteins. Output of the COILS program (http://ulrec3.unil.ch/software/coils_FORM.html) with window width = 28; Lupas *et al.*, 1991).

(Doutriaux *et al.*, 1998) and those of the *UVH1/AtRAD1* (Gallego *et al.*, 2000; Liu *et al.*, 2000) and *UVR2* (Ahmad *et al.*, 1997) genes of *Arabidopsis*.

As reported for the human Rad50 protein following treatment with ionizing radiation (Dolganov *et al.*, 1996), the steady-state level of *AtRAD50* mRNA does not change in cultured cells in response to exposure to the radiomimetic compound MMS (data not shown). This contrasts with the situation reported for mouse *RAD50* gene expression, which does increase after treatment of NIH-3T3 cells with MMS (Kim *et al.*, 1996).

Identification and characterization of a rad50 *mutant of* Arabidopsis

Based on our *AtRAD50* sequence, we designed oligonucleotides and screened the Versailles *Arabidopsis* T-DNA insertion mutant collection (Bechtold *et al.*, 1993; Bouchez *et al.*, 1993) using PCR as described by Gaelen *et al.* (2000). From this screen we identified a single plant containing a T-DNA insertion in the *RAD50* locus. The mutant plant is fully sterile, producing numerous flowers and small, empty siliques (Figure 3).

The mutant phenotype and the kanamycin resistance marker of the inserted T-DNA co-segregate, with selfed heterozygotes producing progeny with the 3 : 1 ratio expected for a single-locus insertion (128 non-mutant : 38 mutant; χ^2, 1 df = 0.39). Southern analysis has confirmed that the T-DNA insertion is present at a single locus (not shown), and PCR results indicate the single-copy nature of the insertion (see the size of the amplified fragment present in the homozygous plants, Figure 4). Molecular analysis shows that the 38 plants presenting the sterility phenotype are, as expected, homozygous for the T-DNA insertion. Thus there is clear linkage between the T-DNA insertion and the sterility phenotype of the mutant *rad50* allele, which is recessive. Furthermore, we have also observed a sterility phenotype in plants expressing *AtRAD50* antisense RNA (not shown). These results suggest a role for the Rad50 protein in meiosis in plants which is consistent with that known in yeast. Due to the cell-lethal phenotype of the *rad50* null mutant in mouse, this constitutes the first implication of *RAD50* in meiosis in a metazoan organism.

Mutant plants which have been germinated on agar medium before being transferred to soil are reduced in size in comparison to their non-mutant siblings; however this

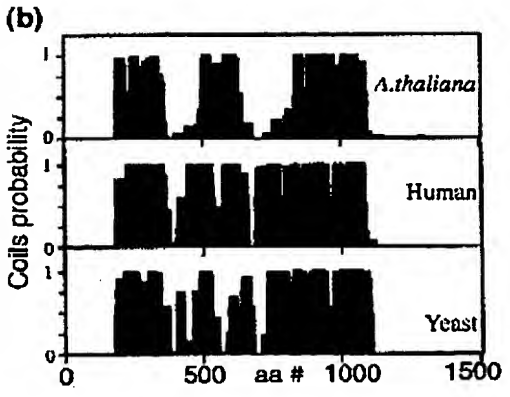

Figure 1b. Legend on facing page.

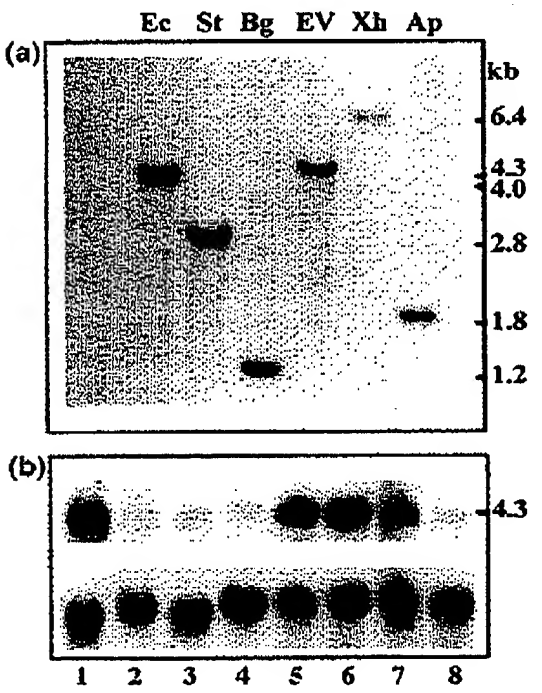

Figure 2. Southern and Northern analysis of *AtRAD50*.
(a) Southern analysis of DNA from *Arabidopsis* cell culture probed with the *AtRAD50* genomic probe. From left to right: *EcoR1*-, *Sty1*-, *Bgl*II-, *EcoRV*-, *Xho*I- and *Apa*I-digested DNA. Sizes of the detected fragments are shown to the left.
(b) Northern analysis of total *Arabidopsis* RNA from different tissues, probed with the 3′ half of the *AtRAD50* cDNA (upper) and the 18S rDNA probe as a loading control (lower). Lanes: 1, suspension cells; 2, young rosette leaves grown for 3 weeks; 3, leaves from stems of mature flowering plants; 4, rosette leaves from non-flowering plants grown for 6 weeks with short daylength (8 h light period); 5, mature flowers; 6, flower buds; 7, roots; 8, stems.

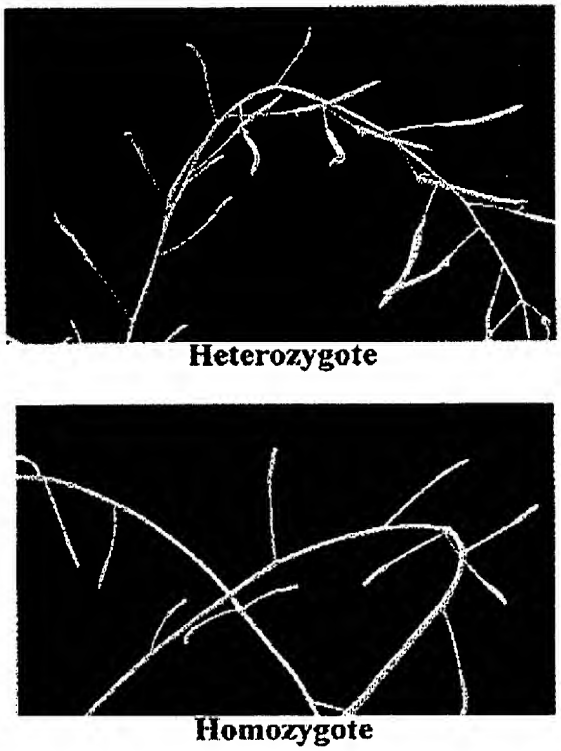

Figure 3. The *rad50* mutant is sterile.
Photographs of 7-week-old mutant (*rad50/rad50*) and heterozygote (*rad50/RAD50*) plants. The mutant plants produce many flowers and small, empty siliques.

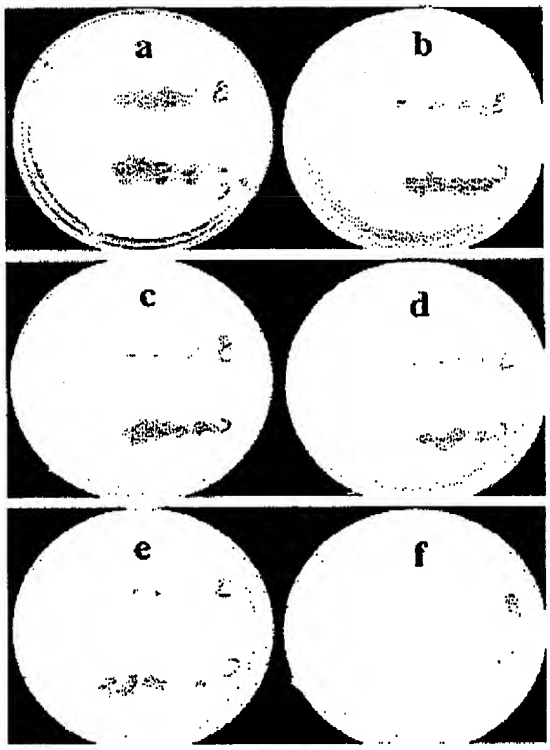

Figure 6. MMS sensitivity of *rad50* mutant cells.
Mutant (*rad50/rad50* upper on each plate) and heterozygote (*RAD50/rad50* lower on each plate) suspension cell lines grown in Petri dishes on agar medium containing different concentrations of MMS: (a) no MMS; (b) 0.0033%; (c) 0.0067%; (d) 0.01%; (e) 0.0133%; (f) 0.0167% v/v MMS.

phenotype is not seen if seeds are germinated directly in soil in the greenhouse. This growth difference is seen whether or not antibiotic (kanamycin) is included in the agar medium, and is thus not an artefact caused by the antibiotic. Further studies will be needed to determine the exact origin of this facultative growth defect.

The mutated *rad50* gene and its inserted T-DNA were PCR-amplified, cloned, and the plant DNA/T-DNA junctions sequenced to determine the exact position and structure of the insertion (Figure 5). The T-DNA is inserted into the coding sequence of the gene and causes disruption of the *RAD50* ORF in the 21st exon, after the 1050th codon (of 1316). Thus any mutant protein produced would lack the 266 C-terminal amino acids of the wild-type protein.

The inserted T-DNA has a small, 141 nucleotide, inverted duplication of the LB sequence upstream of the RB, and has lost the first 10 nucleotides following the RB nick site. The inverted orientation of this LB DNA implies the participation of a replicated T-DNA molecule in the origin of this sequence, and that it is not simply the result of ligation-derived concatamers of the single-stranded transforming T-DNA molecules. The beginning of the insertion (inverted LB sequence) has a 12 bp (with three mismatches) homology to the *RAD50* sequence at that position. The LB junction is much simpler, with the T-DNA sequence ending 16 nt downstream of the LB nick site, followed by the insertion of two As prior to resumption of the *RAD50* gene sequence. The insertion caused an 18 bp deletion (not including the 12 bp homology, above) of *RAD50* sequence. Neither of the two junctions occurs exactly at the nick site of the corresponding border sequence. We have previously seen similar structures at T-DNA insertion sites (unpublished results), and the mechanism of T-DNA integration has been reviewed by Tinland (1996). The systematic sequencing of the DNA flanking T-DNA insertions of the Versailles mutant collection is currently under way, and understanding of these events will greatly benefit from this rich source of data.

The Arabidopsis rad50 *mutant is hypersensitive to MMS*

In yeast cells, mutation of the *RAD50* gene causes hypersensitivity to X-ray irradiation and to particular radiomimetic DNA-damaging chemicals such as MMS. However, due to the cell-lethality of *rad50* mutants in mouse, a clear demonstration of this radiosensitivity has not previously been possible in a metazoan organism (see Luo *et al.*, 1999 for work on gamma-irradiated early mouse blastocyst explants).

We and colleagues (J. Paszkowski, personal communication) have observed that the lethal effects of MMS on *Arabidopsis* seedlings are dependent on the size of the plants tested. Non-ambiguous determination of the MMS concentration needed to kill a given plant line (or other multicellular organism) is thus complicated by questions of the size of plants used. In order to avoid this problem, we therefore tested MMS sensitivity on cell lines derived from these plants. In this way, very small clumps (microcalluses) of growing cells are exposed to MMS, and a much clearer determination of dose-dependent MMS sensitivity is possible. Callus was induced on young leaf tissue, and suspension cultures initiated using standard protocols (see Experimental procedures). Suspension cells in liquid culture were pipetted onto solid growth media with or without different concentrations of MMS, and their growth scored visually 2–3 weeks later (Figure 6). In the absence of MMS the *rad50* mutant and heterozygote cell lines had both grown to similar extents, whilst the *rad50* mutant cells showed a clear hypersensitivity to MMS relative to the heterozygote cell line. We have also observed MMS sensitivity in mutant cell lines generated via the antisense approach (data not shown). These data suggest that the Rad50 protein plays a role in DSB repair in plant cells, as is the case in yeast.

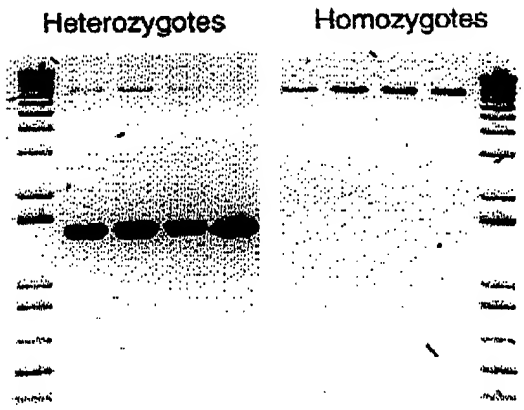

Figure 4. The *rad50* mutant locus contains a single T-DNA insertion.
PCR of individual plants segregating *RAD50*. Total DNA was amplified with a pair of primers in the *RAD50* sequence, spanning the T-DNA insertion site (5'-GAGCTGTGAAGCTAGAAAGAATGAACTTGCAGGTG; 5'-CCCATCCAGGTTTGTAGTTG). Homozygote plants show only the 8 kb PCR product expected for a single-copy T-DNA insertion, while heterozygotes also show the expected 1.5 kb band for the wild-type *RAD50* gene.

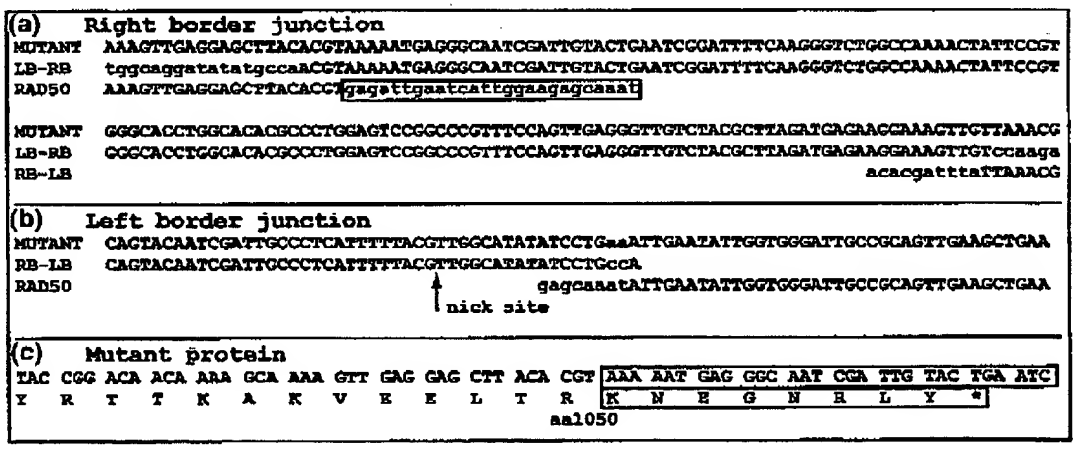

Figure 5. Sequences of junctions of the inserted T-DNA.
RAD50 junctions (a,b) and the predicted effect on the Rad50 protein (c). The mutant *rad50* genomic DNA sequence is aligned with the sequences of the ends of the T-DNA used to generate the *Arabidopsis* mutants, and with the *RAD50* genomic sequence. *RAD50* sequence deleted at the point of the insertion is boxed (a) and the RB-LB sequence shown begins immediately after the RB nick (b). In frame coding DNA (upper) and protein (lower) sequence added to the mutant rad50 open reading frame by the insertion is shown boxed in (c).

The Arabidopsis rad50 *mutant is viable*

To our knowledge, the only other report of a *rad50*⁻ mutant in a metazoan organism describes a 'cell-lethal' phenotype in a mouse *rad50*⁻ mutant (Luo *et al.*, 1999). These authors were unable to isolate homozygous *rad50*⁻ mutants of mouse ES cells in culture, and ascribed the early embryonic lethality of homozygous *rad50/rad50* mice to a gradual cession of proliferation of the embryonic cells. A differential radiosensitivity was also seen in mutant blastocyst explants, and the authors tentatively concluded that the cell lethality is caused by the accumulation of unrepaired and/or misrepaired DNA DSBs, indicating involvement of mouse Rad50 protein in DSB repair/signalling.

We show here that the *Arabidopsis rad50*⁻ mutant is hypersensitive to the radiomimetic agent MMS, and thus presumably defective in DSB repair; however we have no evidence for a strong effect on the proliferative capacity of *rad50*⁻ *Arabidopsis* cells. Under certain growth conditions the mutant plant is stunted, but the facultative nature of this phenotype clearly indicates the lack of a basic problem at the cell division level. As the mutant plant is fully sterile, effects on cellular proliferative capacity can only be studied in the context of a single plant generation. Thus we have derived mitotic cell cultures from the mutant plants in order to be able to study long-term effects. These cells grow, and we are undertaking further analysis on the involvement of the *RAD50* function in DNA repair/recombination/cell proliferation in plant cells.

As the *rad50* mutation in our mutant is caused by a DNA insertion into the coding sequence of the *AtRAD50* gene, we cannot exclude that a truncated protein is produced in the mutant cells, which would thus not be true 'null' mutants. Preliminary data using antibodies to a C-terminal fragment of the AtRad50 protein shows the absence of the AtRad50 protein band on Western blots of protein from the mutant cells (S. Daoudal and C.I.W., unpublished results); however these antibodies may not recognize a C-terminus-truncated form of the protein. It is thus formally possible that this putative truncated protein is sufficient to fulfil the function of the Rad50 protein in cell viability. However, given the clear phenotypes of the mutant for meiosis and MMS sensitivity, we think it extremely unlikely that the viability of the rad50⁻ mutant cells and plants can be explained in this way. We note that the question of whether a given mutation is null may be posed for any insertional mutation that does not involve full deletion of the open reading frame of the gene in question (i.e. including all insertional mutations). Whether or not our mutant is 'null' does not alter our conclusions on the role of the AtRad50 protein in *Arabidopsis* and the phenotypes of the T-DNA insertion mutant described here. Full resolution of this question must await the identification of a full *Atrad50* deletion mutant.

In conclusion, the plant Rad50 function appears to be analogous to that of yeast. Given the difficulties encountered in isolating and studying the mouse *rad50*⁻ mutant, we believe that further study of *rad50*⁻ mutant plant cells will permit us to clarify the role of this polyvalent protein and its multiple roles in the maintenance of genome integrity in both plants and animals.

Experimental procedures

Growth of cell suspensions and plants

The *Arabidopsis thaliana* cell suspension (T87) was established by Axelos *et al.* (1992). Cells were grown in Gamborg's B-5 medium (Sigma #G5893, St Quentin Fallavier, France) supplemented with 30 g l^{-1} sucrose and 200 mg l^{-1} naphthalene acetic acid (NAA) on a rotating platform (120 rpm) at 22°C with 16 h light/8 h dark. Cells in liquid culture were subcultured at weekly intervals. Bacto-agar (Difco, Detroit, MI, USA) was included in the cell-suspension medium at 0.8% w/v for solid media. *Arabidopsis thaliana* (Columbia) seeds were sown directly into damp compost and germinated in a greenhouse under white light (16 h light/8 h dark). For the isolation of root tissue, plants were grown aeroponically under the same conditions.

Callus cultures were derived from homozygous *rad50/rad50* and heterozygous *rad50/RAD50* plants using standard techniques as follows (J. Lucht and B. Hohn, personal communication). Leaves of young germinating plants were surface-sterilized in 0.5% sodium hypochlorite, 0.05% Tween-80 for 10 min at RT and rinsed several times with sterile water. The leaves were then cut up with a sterile scalpel and placed on callus induction medium (CIM) agar for 1 week (22°C, 16 h light). The leaf/callus was then transferred to shoot induction medium (SIM) agar medium, and after 2–3 weeks successfully growing green callus was transferred to fresh SIM medium (either solid or liquid) and then maintained on this medium by regular subculture. CIM and SIM media were prepared as for the Gamborg's B-5 medium (above), except that the hormones (Sigma) differ: CIM, 1 mg l^{-1} 2,4-dichlorophenoxyacetic acid, 0.2 mg l^{-1} kinetin; SIM, 0.1 mg l^{-1} NAA, 1 mg l^{-1} 6-benzylaminopurine.

Isolation of the AtRAD50 *cDNA and genomic DNA clones*

Using poly(A)+ RNA prepared from the cell-suspension culture of *Arabidopsis*, the complete *AtRAD50* cDNA was isolated using the marathon RACE PCR kit following the manufacturer's instructions (Clontech).

The genomic DNA clone was isolated from a genomic DNA library constructed with Sau3A1 partially digested DNA from the cell suspension cloned into lambda FIXII (Stratagene Inc., La Jolla, CA, USA) following the manufacturer's protocol.

Southern analysis

Genomic DNA was isolated from cell-suspension cultures following the method of Dallaporta *et al.* (1983). DNA (3 μg) was digested with 50 units of the relevant restriction enzyme in a volume of 100 μl for 16 h at the recommended temperature.

Digested samples were phenol/chloroform extracted, ethanol precipitated, resuspended in TE and electrophoresed in 0.8% agarose/TAE gels. The gels were capillary blotted to Hybond N+ (Amersham, Orsay, France) positively charged nylon membrane, and hybridized at 62°C to radioactively labelled DNA probes according to Church and Gilbert (1984). Filters were then washed (0.1 × SSC, 0.1% SDS, 62°C) and autoradiographed. Probes were labelled with α-^{32}P dCTP using the Prime-It II kit (Stratagene) according to the manufacturer's instructions.

RNA isolation and Northern analysis

Frozen plant or cell-culture tissue was homogenized in liquid N_2 with a mortar and pestle. Total RNA was then prepared using the Trizol reagent following the manufacturer's instructions (Gibco-BRL, Cergy Pontoise, France). Poly(A)+ RNA was purified from total RNA using the mRNA Direct kit (Dynal Inc., Compiègne, France). For Northern analysis, 30 µg RNA per lane was fractionated on 0.8% agarose/formaldehyde gels, which were blotted, hybridized to radioactively labelled probes, and autoradiographed as for the Southern blots (above).

Callus MMS sensitivity tests

0.5 ml of suspension cells were pipetted onto the surface of agar plates containing solid SIM medium and different concentrations of MMS (Sigma #M4016). The plates were then incubated as described above, and resistance or sensitivity was scored visually 2–3 weeks later. MMS-containing plates were prepared immediately before use. All MMS-contaminated material was quenched after use by soaking in 10% w/v sodium thiosulphate.

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GenBank accession number AF168748.

U.S. Serial No. 09/538,396
Group Art Unit: 1638

APPENDIX D

APPENDIX D - motifs from Example 4, Pfam added to Appendix A (2/14/02)
Docket 1116E
Serial Numb r 09/538,396

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GapWeight: 8 GapLengthWeight: 2
MSF: 1380 Type: P February 11, 2002 15:38 Check: 219 ..

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NM022246aa	Rat Rad50
U66887aa	Mouse Rad50
AAD15407aa	Arabidopsis Rad50
AF168748aa	Arabidopsis Rad50
1116sid2	**SEQ ID NO: 2 Maize Rad50**
X14814aa	Yeast Rad50
Z75312aa	C. elegans Rad50

FORMATTING: [redacted]

MOTIFS: Identified in Example 4 and Pfam analysis
Leucine zipper - Underlined, LZ noted in left margin
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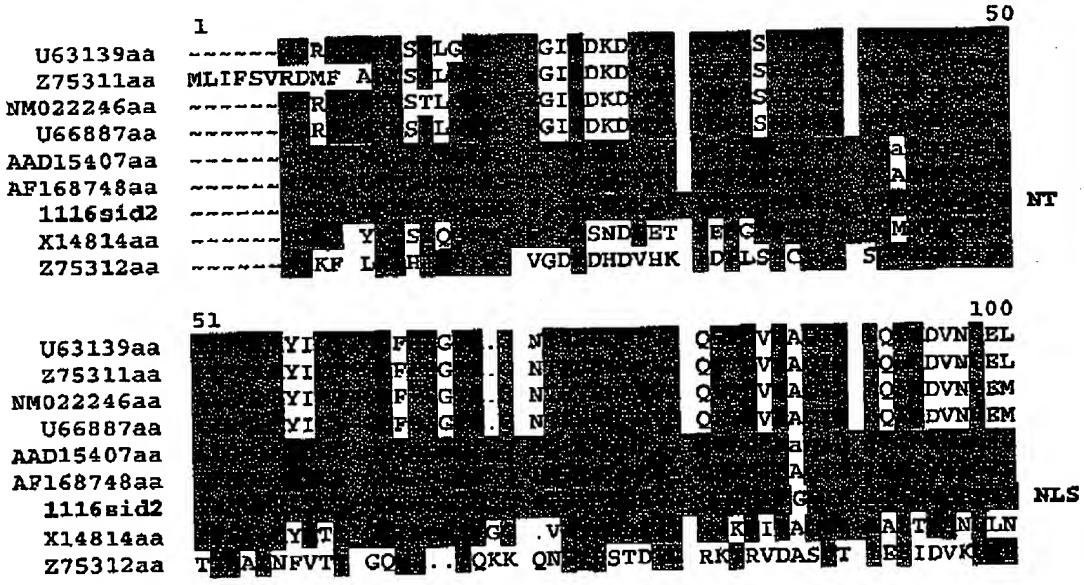
Nuclear localization signal - boxed, NLS in left margin
Rad50 Zn-Hook - Double-underlined, ZN noted in left margin



APPENDIX D – motifs from Example 4, Pfam added to Appendix A (2/14/02)
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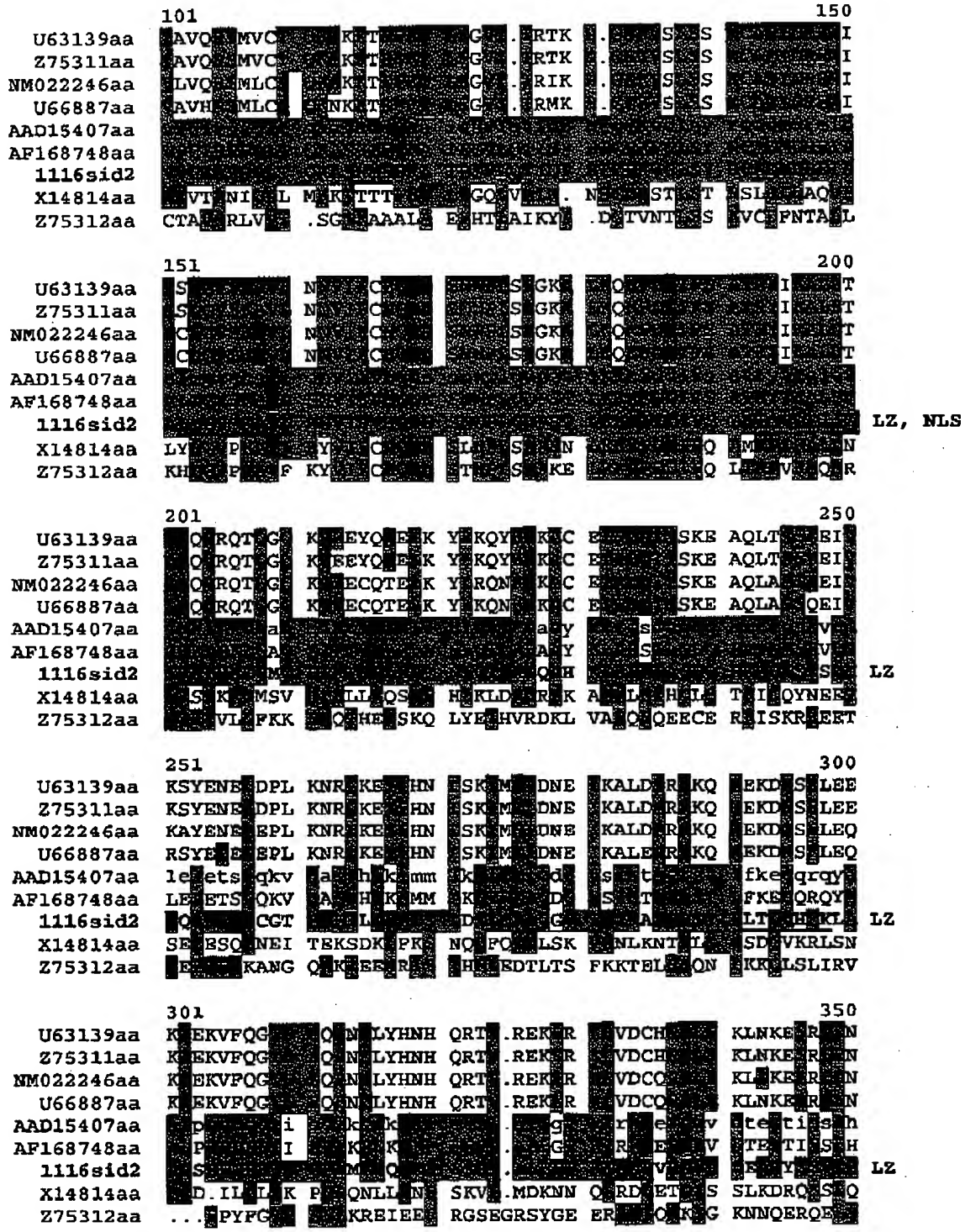
Serial Number 09/538,396



APPENDIX D – motifs from Example 4, Pfam added to Appendix A (2/14/02)
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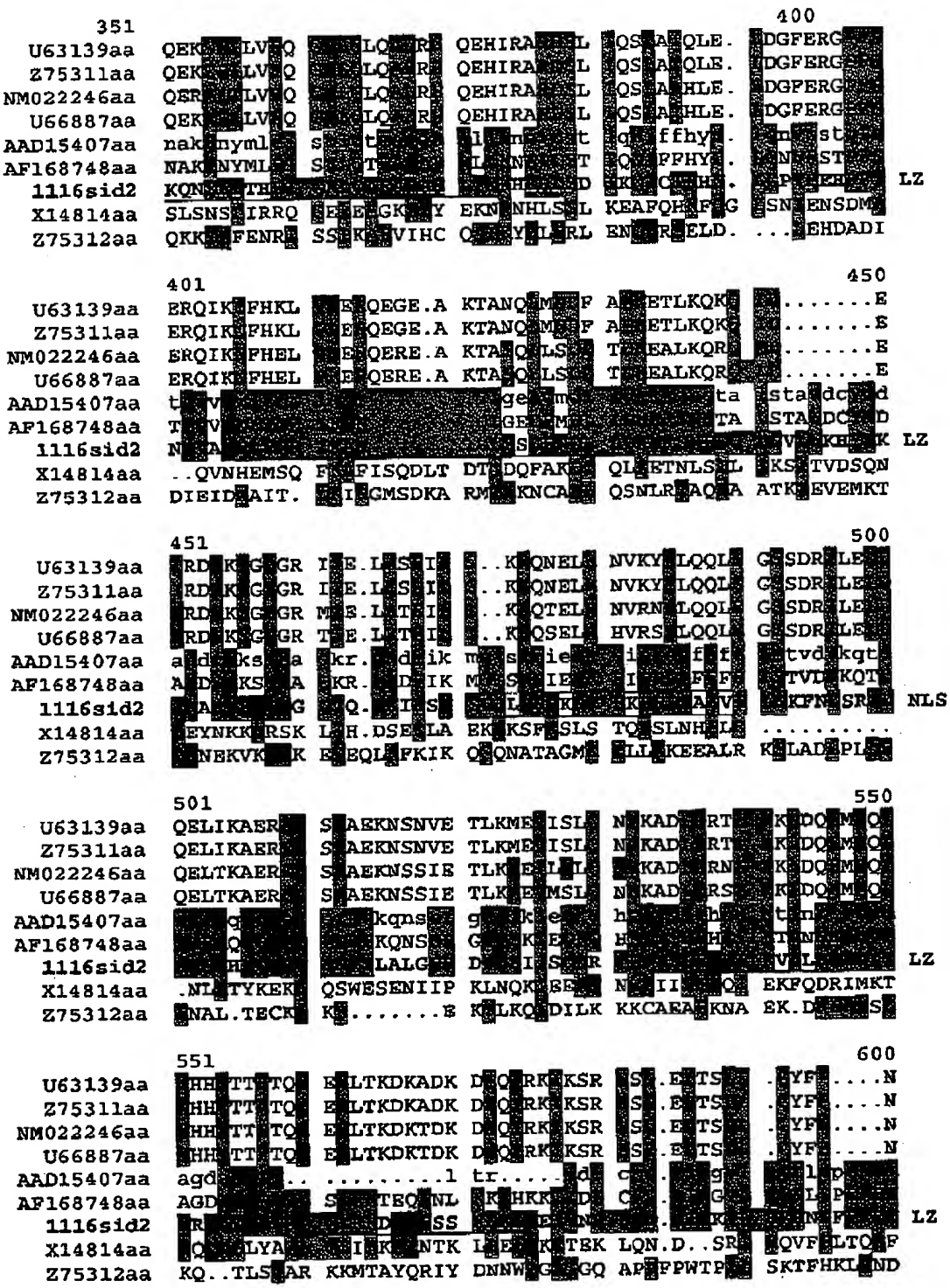

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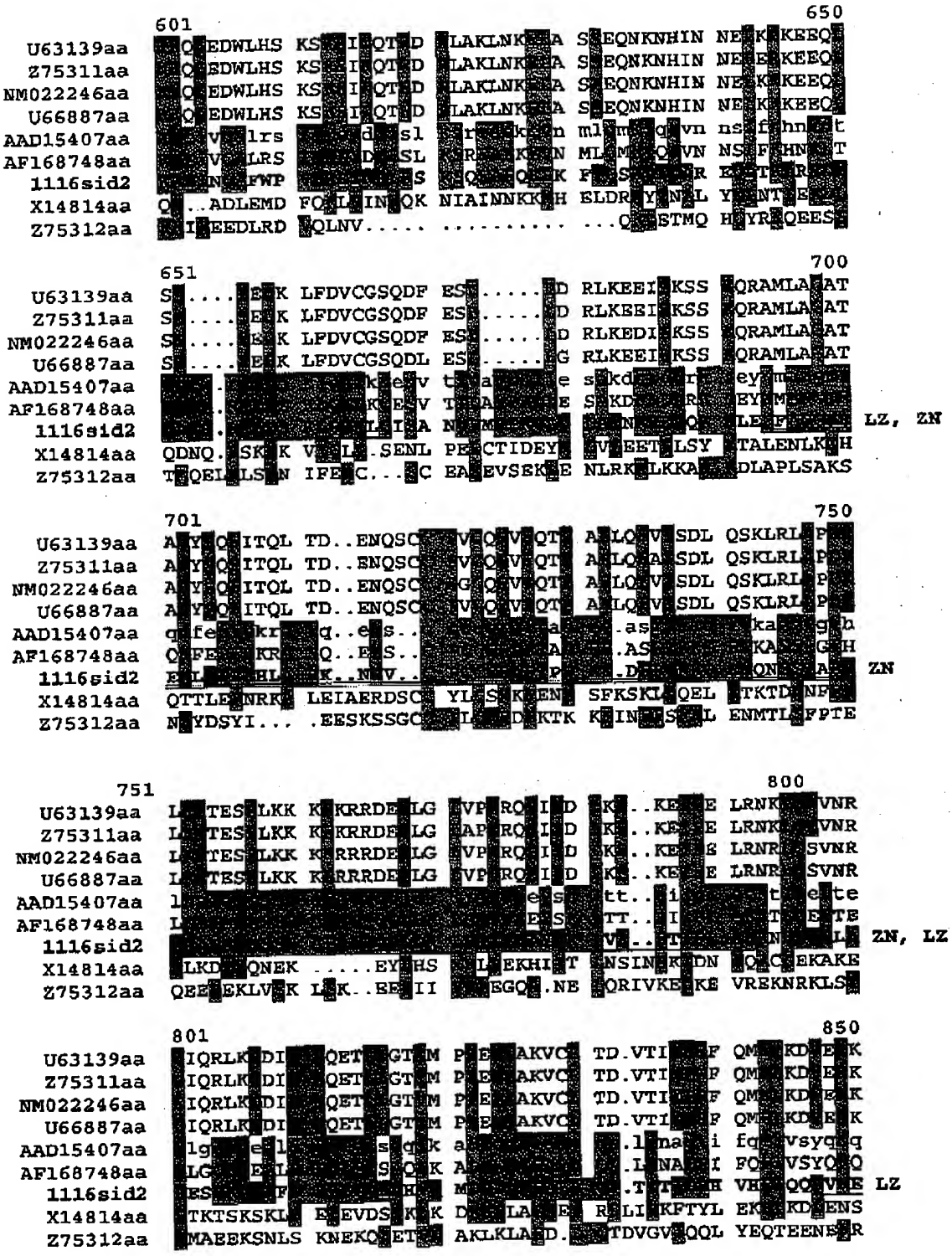
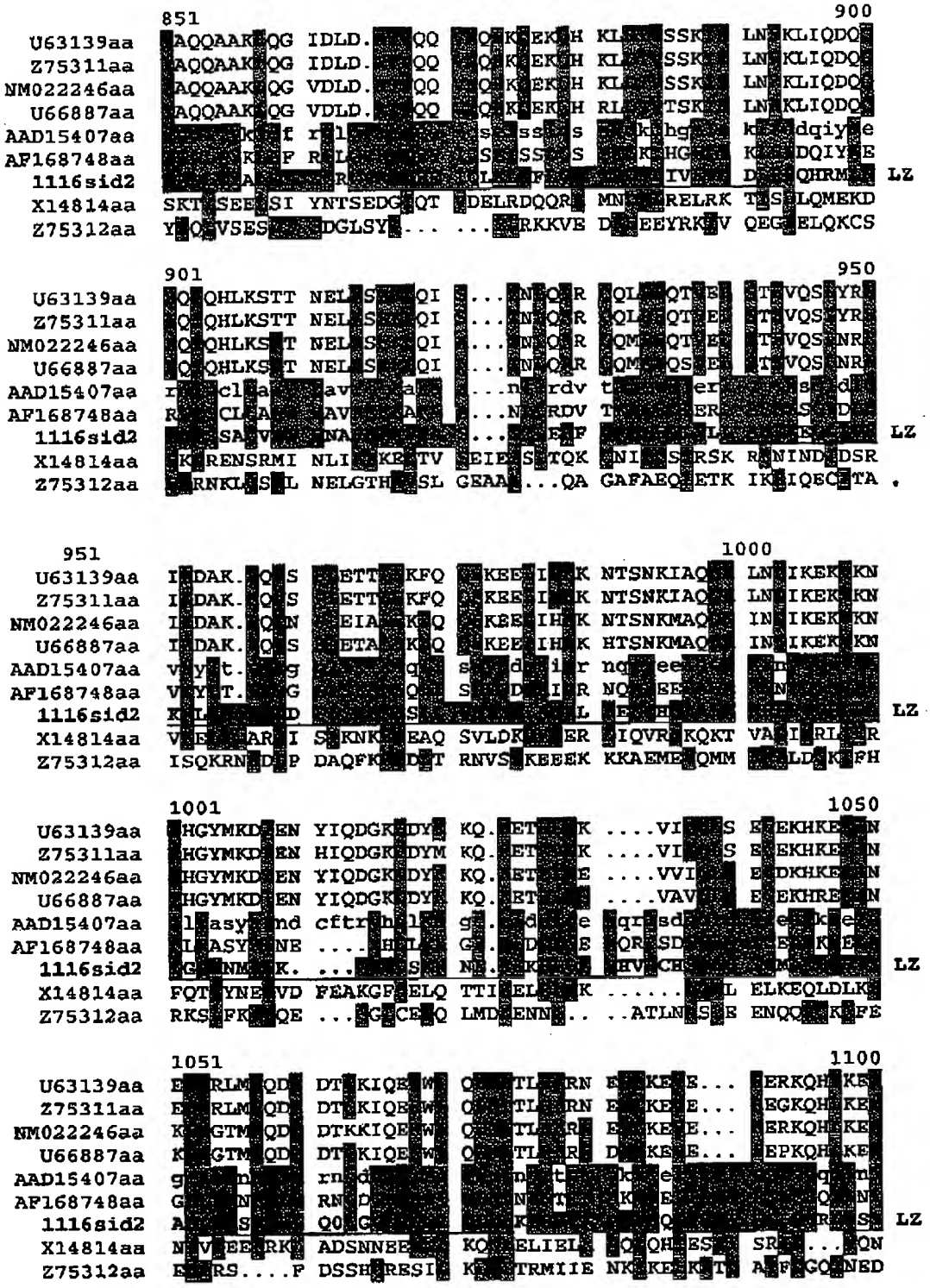
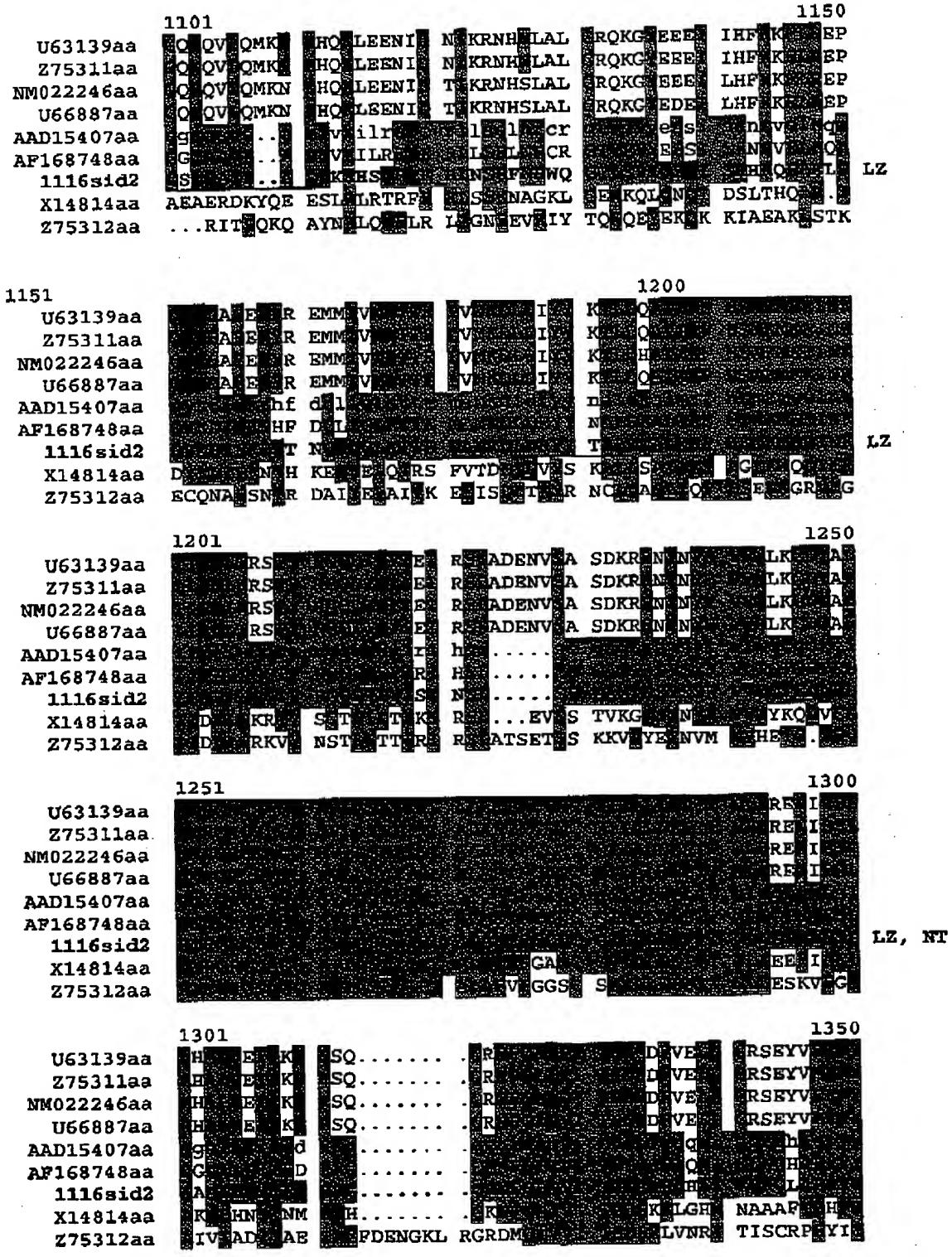
Docket 1116E
Serial Number 09/538,396



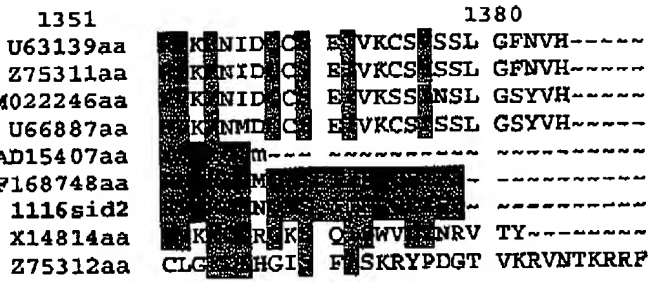
APPENDIX D – motifs from Example 4, Pfam added to Appendix A (2/14/02)
Docket 1116E
Serial Number 09/538,396



APPENDIX D – motifs from Example 4, Pfam added to Appendix A (2/14/02)
Docket 1116E
Serial Number 09/538,396



APPENDIX D – motifs from Example 4, Pfam added to Appendix A (2/14/02)
Docket 1116E
Serial Number 09/538,396



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Analysis Browser: Level Up

Report for 1116E.rad50.sid2 (Protein) Update

Description 1116E_rad50_sid2 Edit

Function

DNA repair protein RAD50 (153 kDa protein).

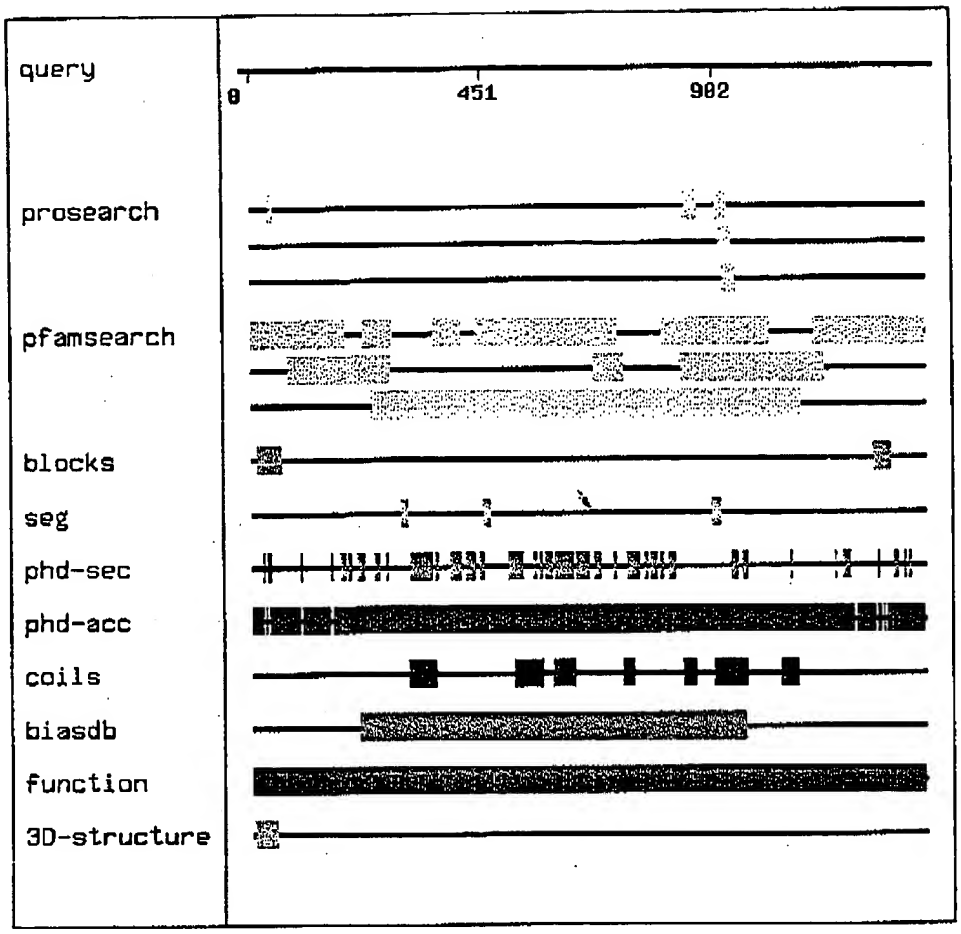
Direct assignment of functionality by homology to swiss|P12753|RA50_YEAST

in region 1 to **1314** for overall length of **350** (99% of query, 375% of hit, see the alignment).

Functional class Transcription

Extracted keywords ATP-binding, Coiled coil

Features Summary



Homologies			All BLAST hits
	Protein	30 clear homologs	All protein BLAST hits
	ESTs	170 homologs	All EST BLAST hits
	Patents	53 homologs	All patent hits
General	**Gene name**		
	Molecular weight	152.50 kD	
	Sequence length	1316	
	Isoelectric point	6.04	
	Predicted cellular localisation (PHD and PreLoc)	nuclear (94.7 %)	
3D Structure	**3D structure inferred by unlikely homology from residues 6 to 48 in 1E69-A**		

	View	alignment
	pdb|1E69|1E69-A	structure
Phylogeny	**Distribution**	34 species extracted from 175 homologous sequences. Species
	Taxa	Chordata, Eukaryotae, Fungi, Planta
	Model organisms	*Arabidopsis thaliana, Caenorhabditis elegans, Drosophila melanogaster, Homo sapiens, Mus musculus, Saccharomyces cerevisiae*
Features	**Coiled coil region**	from **311** to **336**, from **341** to **361**, from **517** to **538**, from **541** to **569**, from **592** to **633**, from **727** to **747**, from **844** to **867**, from **903** to **928**, from **930** to **969**, from **1036** to **1066** detected by [Coils]
	Low complexity region	from **296** to **307**, from **456** to **469**, from **902** to **915** detected by [seg]
	E-rich region	from **212** to **964** detected by [biasdb]
	No significant hits detected by	[Phd-tm]
Patterns	**SMC family, C-terminal domain - region**	from residue **1100** to **1314**. Source: [pfamsearch] . Quality: (E=0.52)
	ABC transporter - region	from residue **1141** to **1296**. Source: [pfamsearch] . Quality: (E=0.19)
	Intermediate filament protein - region	from residue **840** to **1118**. Source: [pfamsearch]
	Myosin tail - region	from residue **237** to **1075**. Source: [pfamsearch]
	Uncharacterized ACR, COG1579 - region	from residue **804** to **1015**. Source: [pfamsearch]
	KE2 family protein - region	from residue **891** to **983**. Source: [pfamsearch]
	Rad50 zinc hook motif region	from residue **673** to **726**. Source: [pfamsearch] . Quality: (E=9.9e-06)
	Protein of unknown	from residue **441** to **717**. Source: [pfamsearch]

function, DUF259 - region	
Late embryogenesis abundant (LEA) group - region	from residue **548** to **625.** Source: [pfamsearch]
Rad50 zinc hook motif region	from residue **360** to **413.** Source: [pfamsearch]
Heat shock protein 9 / 12 - region	from residue **219** to **275.** Source: [pfamsearch]
Poly(A) polymerase central domain - region	from residue **75** to **273.** Source: [pfamsearch]
Sigma-70, non-essential region - region	from residue **112** to **260.** Source: [pfamsearch]
RecF/RecN/SMC N terminal domain - region	from residue **2** to **182.** Source: [pfamsearch] . Quality: (E=0.48)
LEUCINE_ZIPPER region	from residue **922** to **944.** Source: [prosite]
	from **915** to **937.** Source: [prosite]
	from **908** to **930.** Source: [prosite]
	from **849** to **871.** Source: [prosite]
ATP_GTP_A region	from residue **34** to **42.** Source: [prosite]
No significant hits found in	[blocks database]

Comment No comment section. Edit

Completed Tasks

Start Time	User	Comment	Output	Interactiv
04.04.2003, 16:44:19	dressvm		bioSCOUT_default details...	

Permissions Edit

Alert Jobs New

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Alignment: 1116E.rad50.sid2 - pdb|1E69|1E69-A

BLASTP - alignment of 1116E.rad50.sid2 against pdb|1E69|1E69-A

chromosome segregation smc proteinfragment: smc fusion of the n- and c-terminal globular domains residues 1-152 and 1023-1164;

- This hit is scoring at : 0.56 (expectation value)
- Alignment length (overlap) : 43
- Identities : 44 %
- Scoring matrix : BLOSUM62 (used to infer consensus pattern)
- Database searched : nrdb

```
Q:      6 KMLIKGIRSFDPDNKNVITFFKPLTLIVGPNGAGKTTIIECLK      48
          K:.:KG.:SF.  ..::I F . :T.IVGPNG:GK:.II:.:K
H:      5 KLYLKGFKSFG--RPSLIGFSDRVTAIVGPNGSGKSNIIDAIK      45
```

Legend of Alignment

```
:  positive score

.  score between -2 and 0
```

Please report problems and feedback concerning bioSCOUT through the support interface.

```
HEADER    CHROMOSOME SEGREGATION                  09-AUG-00   1E69
TITLE     SMC HEAD DOMAIN FROM THERMOTOGA MARITIMA
COMPND    MOL_ID: 1;
COMPND   2 MOLECULE: CHROMOSOME SEGREGATION SMC PROTEIN;
COMPND   3 CHAIN: A, B, C, D, E, F;
COMPND   4 FRAGMENT: SMC FUSION OF THE N- AND C-TERMINAL GLOBULAR
COMPND   5 DOMAINS RESIDUES 1-152 and 1023-1164;
COMPND   6 ENGINEERED: YES
SOURCE    MOL_ID: 1;
SOURCE   2 ORGANISM_SCIENTIFIC: THERMOTOGA MARITIMA;
SOURCE   3 ATCC: DSM3109;
SOURCE   4 CELLULAR_LOCATION: CYTOSOL;
SOURCE   5 EXPRESSION_SYSTEM: ESCHERICHIA COLI;
SOURCE   6 EXPRESSION_SYSTEM_STRAIN: C41(DE3);
SOURCE   7 EXPRESSION_SYSTEM_CELLULAR_LOCATION: CYTOSOL;
SOURCE   8 EXPRESSION_SYSTEM_VECTOR_TYPE: PLASMID;
SOURCE   9 EXPRESSION_SYSTEM_PLASMID: PHIS17
KEYWDS    SMC, STRUCTURAL MAINTENANCE OF CHROMOSOMES, COILED COIL
EXPDTA    X-RAY DIFFRACTION
AUTHOR    J.LOWE,S.C.CORDELL,F.VAN DEN ENT
REVDAT   2   27-MAR-01 1E69    1       JRNL
REVDAT   1   09-AUG-00 1E69    0
JRNL        AUTH   J.LOWE,S.C.CORDELL,F.VAN DEN ENT
JRNL        TITL   CRYSTAL STRUCTURE OF THE SMC HEAD DOMAIN: AN ABC
JRNL        TITL 2 ATPASE WITH 900 RESIDUES ANTIPARALLEL COILED-COIL
JRNL        TITL 3 INSERTED
JRNL        REF    J.MOL.BIOL.                    V. 306    25 2001
JRNL        REFN   ASTM JMOBAK  UK ISSN 0022-2836
```

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Summary

Searched query 1116E.rad50.sid2 against PFAM database.

Hit	Score	Expect	Description	Q from	Q to	Method
pfam|hmm|Rad50_zn_hook. alignment	32.5	9.9e-06	Rad50 zinc hook motif	673	726	HMMPFAM
pfam|hmm|Rad50_zn_hook. alignment	4.2	1.8	Rad50 zinc hook motif	360	413	HMMPFAM
pfam|hmm|HSP9_HSP12. alignment	-11.4	4.1	Heat shock protein 9 / 12 -	219	275	HMMPFAM
pfam|hmm|LEA_1. alignment	-14.3	5.6	Late embryogenesis abundant (LEA) group -	548	625	HMMPFAM
pfam|hmm|KE2. alignment	-19.0	5.6	KE2 family protein -	891	983	HMMPFAM
pfam|hmm|PAP_central. alignment	-45.9	7.6	Poly(A) polymerase central domain -	75	273	HMMPFAM
pfam|hmm|ABC_tran. alignment	-47.7	0.19	ABC transporter -	1141	1296	HMMPFAM
pfam|hmm|SMC_N. alignment	-69.5	0.48	RecF/RecN/SMC N terminal domain -	2	182	HMMPFAM
pfam|hmm|DUF164. alignment	-90.1	2.8	Uncharacterized ACR, COG1579 -	804	1015	HMMPFAM
pfam|hmm|SMC_C. alignment	-116.7	0.52	SMC family, C-terminal domain -	1100	1314	HMMPFAM
pfam|hmm|sigma70_ner. alignment	-122.2	4.9	Sigma-70, non-essential region -	112	260	HMMPFAM

pfam|hmm|DUF259 . alignment	- 137.1	9.3	Protein of unknown function, DUF259 -	441	717	HMMPFAM
pfam|hmm|filament . alignment	- 203.9	5.4	Intermediate filament protein -	840	1118	HMMPFAM
pfam|hmm|Myosin_tail . alignment	- 555.1	9.8	Myosin tail -	237	1075	HMMPFAM

New Task | Rename Sequences

Please report problems and feedback concerning bioSCOUT through the support interface.

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Alignment: 1116E.rad50.sid2 - pfam|hmm|Rad50_zn_hook

HMMPFAM - alignment of 1116E.rad50.sid2 against pfam|hmm|Rad50_zn_hook

Rad50 zinc hook motif

- This hit is scoring at : 4.2
- Scoring matrix : BLOSUM62 (used to infer consensus pattern)

```
Q:    360 AHLTMKHERDSDIKNICTKHNL--GPVPEHPF-TNDVAMNLTNRIKARLSSLENDLL     413
          . L..:... ... . ..:..     PV  ... T.: ...L....K:.L..L..:L
H:      1 galesekaelkkaieeleeeesscCPvCgReLgteeekkelikeykseldrlpeelk      57
```

HMMPFAM - alignment of 1116E.rad50.sid2 against pfam|hmm|Rad50_zn_hook

Rad50 zinc hook motif

- This hit is scoring at : 32.5
- Scoring matrix : BLOSUM62 (used to infer consensus pattern)

```
Q:    673 NFANGMREMLAPFEHLARKNHV--CPCCERAF-TPDEEDEFVKKQRMQNSSTAERSK     726
          . ... :. L..  . ..:..   CP.C R.. T.:E:.E.:K:.:.: ....E. K
H:      1 galesekaelkkaieeleeeesscCPvCgReLgteeekkelikeykseldrlpeelk      57
```

Legend of Alignment

```
:  positive score

.  score between -2 and 0
```

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Alignment: 1116E.rad50.sid2 - pfam|hmm|HSP9_HSP12

HMMPFAM - alignment of 1116E.rad50.sid2 against pfam|hmm|HSP9_HSP12

Heat shock protein 9 / 12 -

- This hit is scoring at : -11.4
- Scoring matrix : BLOSUM62 (used to infer consensus pattern)

```
Q:    219 --DQAHKLRENIAQDQEKSDASKSQMEQLKEKICGTEREILQMETSLDELRRLQGQIDI    275
            D A.K   . A::: ..D:SKS..EQ:KEK:...  ::. . TS D: ..:Q . D
H:      1 MSDlaRKdFgeKakEklTPDSsKSTaEqvKEkvTDklDkvAgkvtsdddKStvQkAhDk     59
```

Legend of Alignment

: positive score

. score between -2 and 0

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Alignment: 1116E.rad50.sid2 - pfam|hmm|LEA_1

HMMPFAM - alignment of 1116E.rad50.sid2 against pfam|hmm|LEA_1

Late embryogenesis abundant (LEA) group -

- This hit is scoring at : -14.3
- Scoring matrix : BLOSUM62 (used to infer consensus pattern)

```
Q:     548 LESSKDKLNEIVNEHKDKIKKVLrgrnpFEKDMKKEINQAFWPVDKEYNELRSKSQEAEQ
           ::S:K:K:::.:.:. K:K:. .        :.D K.E  .A  . :KE. . R.K::EA:.
H:       1 MqSaKEKisnmAstAKekmditK.....AkadEKaEkatARtkeEkelAhqrkkAKeAqA

           ELKFTQSKVTDAREQLTK      625
           E:.. ::K...A.E: :.
           eMdlheaKAehaaekesa       73
```

Legend of Alignment

```
:  positive score

.  score between -2 and 0
```

Please report problems and feedback concerning bioSCOUT through the support interface.

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Alignment: 1116E.rad50.sid2 - pfam|hmm|KE2

HMMPFAM - alignment of 1116E.rad50.sid2 against pfam|hmm|KE2

KE2 family protein -

- This hit is scoring at : -19.0
- Scoring matrix : BLOSUM62 (used to infer consensus pattern)

```
Q:     891 ASSILERFQKSEEELVLLAEEKEQLIVEKKLLEESLDPLSKEKE3----------LL-Q
           ...:L.:.Q: :::L  :..:K.QL  : K .E  L:.L.K .E.          :  Q
H:       1 vqellaklqqlqqqlekvmtqkaqlerqLkEaelvleELekldeDtkVYklVGkvLVkvq

           EYNALKQKLDEEYHQLAERKREFQQELDALGR----LNMKIKGYLDSKK     983
           : ...:.:L:E...QL.E. :..::: : L :   L. K:: .L.S..
           dkeeardeLeerleqleeeiktLekqeeylekeleeleeklqellqsaa     109
```

Legend of Alignment

```
:  positive score

.  score between -2 and 0
```

Please report problems and feedback concerning bioSCOUT through the support interface.

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Alignment: 1116E.rad50.sid2 - pfam|hmm|PAP_central

HMMPFAM - alignment of 1116E.rad50.sid2 against pfam|hmm|PAP_central

Poly(A) polymerase central domain -

- This hit is scoring at : -45.9
- Scoring matrix : BLOSUM62 (used to infer consensus pattern)

```
Q:      75          xsNWPLQDPSTLKKKFDDIFSAT-RYTKALEVIKK-LMKDQMQEIKTFRLK
                       :P . PSTL :KF  :FS.  R:. . .V: K ::.D.::E ..
H:       1 RFE.radP.lYPnavpstlvekfFlvfsqWlrhnwpnPVlLkeinsdsieernlqvrvRF

                     273

            E.rade     205
```

Legend of Alignment

: positive score

. score between -2 and 0

Please report problems and feedback concerning bioSCOUT through the support interface.

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Alignment: 1116E.rad50.sid2 - pfam|hmm|ABC_tran

HMMPFAM - alignment of 1116E.rad50.sid2 against pfam|hmm|ABC_tran

ABC transporter -

- This hit is scoring at : -47.7
- Scoring matrix : BLOSUM62 (used to infer consensus pattern)

```
Q:    1141 --------------TALDKALMRFHS-------MKM-EEINKII-----KELWQ------
                         :.L K.:. . .        :.  .::::.:       E  :
H:       1 GevlalvGpNGaGKSTLLklisGllppteGtilldGardlsdlsklkerlerlrknigvv

            ----QTY---RGQDIDYISINSDS----EGAGTRSY-----SYRVVMQTGDA--ELEMR-
                ..:   . .  : I:..        .  .R.      : .:: :.G .  .L  R
            fQdptlfpnpeltvreniafglrlslglskdeqrdrlkkagaeelLerlglgydhlldrr

            -GRCSAGQKVlasliiRLALAETFCLNCGILALDEPTTNLDgpnaESLAAALLRIMEARK
             G..S.GQK       R:A:A......  :L.LDEPT..LD    .: .A.LL.::.. :
            pgtLSGGqkQ......RvaiARaLltkpklLlLDEPTagLD....pasraqllellrelr

            GQeNFQLIVITHDERF-AHLIGQRQLAEKY    1296
             Q . .:::ITHD  .  .L..:  :.E.
            qq.ggTvllitHdldlldrlaDrilvledG     199
```

Legend of Alignment

: positive score

. score between -2 and 0

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Alignment: 1116E.rad50.sid2 - pfam|hmm|SMC_N

HMMPFAM - alignment of 1116E.rad50.sid2 against pfam|hmm|SMC_N

RecF/RecN/SMC N terminal domain -

- This hit is scoring at : -69.5
- Scoring matrix : BLOSUM62 (used to infer consensus pattern)

```
Q:      2 STVDKMLIKGIRSFDpdNKNVI-TFFKPLTLIVGPNGAGKTTIIECLKL----SCT-GEL
            . :.:: ::G.:S:   .K.VI .F . .T.IVGPNG:GK:.I:.: .    . :  :L
H:      1 mylkrielegFKSYa..gktvigpFspgFtaIvGPNGSGKSNilDAIlFVLGegrsakkL

          PPNSRSGHTFVH--DPKVAGeteTKGQIKLRFKTAAGKDVV----CIRSFQLTQKASKME
           ... S. ..    ... A.   ...:.:.F...  :::      IR. .L.:: .:.:.
          RaerlsdLIhkgsggkppan...ksAeVtitFdnedkeniselgqhirdgpldeenpevt

          FKAIESV----LQT-----I------NPHTGEK---VCLSYRCADMDREIPALMGVSKAV
          ...          :     I      N .. .K   V   ...A.:D E:..L..  ...
          ItRrvyrlglgdGstSeYyiWknlrlNgkrvtklkevqelLesagIdiElatLangvsky

          ------------------------------------------------------------

          RayaWCFHWDkELiLleqIswRaeYeaLapeiETCqlFLPElLtvSfqrGWeKetdYaEv

          ------------------------------------------LENVIFVHQDE-------
                                                     . ...: Q.E
          LarnFERDkqLgYTqGPqKADLRlRAnGlPVEDvLSRGQLKLknpyfiilQGeyltrekg

          -------SNWPLQDPSTLK-------KKFDDIF-S     182
                 .: . ..P.. :       : .::I  :
          rhClYLvedIasmkPkeRreldeGLlellEEisGt     330
```

Legend of Alignment

: positive score

. score between -2 and 0

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Alignment: 1116E.rad50.sid2 - pfam|hmm|DUF164

HMMPFAM - alignment of 1116E.rad50.sid2 against pfam|hmm|DUF164

Uncharacterized ACR, COG1579 -

- This hit is scoring at : -90.1
- Scoring matrix : BLOSUM62 (used to infer consensus pattern)

```
Q:     804 PTDTIDrHVHEIQQLVKEVEDLEYALDSSgrgVKSLEEIQLELNPLQRTRDTLIVEVDDL
             .:.:. .: :IQ:: KE E LE .:..     K.L::.:  L. L::. :.L  E ::L
H:       1 mknelk.sLvkiqeidkekerLeerikei...pkeLkkakellealkkeveeleqekeel

            RDQHRMLNEDMSSAQVRWHNArEEKVKA-------SSILERFQKSEEELVLLAEEKEQLI
            ::: :.L.:::...: : ..A EEK:..        .:: ...QK:::: V.L.:E EQL
            keevkklekeiqeieekikka.Eekmdeiktqr EYkALerElqkakdkevtlrkeieqle

            VEKKLLEESLDPLSKEKESLLQEYNALKQKLDEEYHQLAERKREFQQELDalgRLNMKIK
             E K :EE.::.L.:E  .  :E... ::::: E..::.E:  E. .: :   .L. K..
            eelkkieeeieelkeeilkqEkeleeeeeeevelEvrkikekvlellskre...elkektd

            GYLDSKKNEKLKELQGRHVL---------CH----SQLQSCMAKQ-QRIS----------
              I S  .. :K. :.  ::         CH    SQ.:: :.K: . I
            edllsfYERiiknkknlviVPiennvCaGChiiLpsqfenkVrkePddivfCPyCSRILY

            AEL    1015
             E
            yee     235
```

Legend of Alignment

`:` positive score

`.` score between -2 and 0

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Alignment: 1116E.rad50.sid2 - pfam|hmm|SMC_C

HMMPFAM - alignment of 1116E.rad50.sid2 against pfam|hmm|SMC_C

SMC family, C-terminal domain -

- This hit is scoring at : -116.7
- Scoring matrix : BLOSUM62 (used to infer consensus pattern)

```
Q:    1100 ISKHKQELKLS------------QYKDIEKRYTNQFLQLKTTEMANKDLDRYYTALDKAL
           :.K. :EL                :Y.::E:RY.. . :L:..E ..K.L .....LDK.
H:       1 lekeikeLgpVlnElqvNlkAieEYaeveeRyeelvekledleeerkkllevieeldkkr

           MRFHSMKMEEINKIIKELWQQ-TyrgqdidyisinsdsegagtrsysyrvvmQTGDAELE
           :. .. ...:INK .KE::Q: T                                GDAEL.
           leeFmeafnkfnknfkevFqeLt.............................gGGdAeLr

           MRG---RCSAGQKVLAS------LIIRL---------ALAETFCLNCG----ILALDEPT
           :..     S:G :: A.        :.L          ALA .F.::       ...LDE .
           LtDpdDPFssGieisArPPgKkwknlelLSGGEKtLtALALlFAIhkykPsPFYvlDEvD

           TNLDGPNAeSLAAALLRimeaRKGQENFQLIVITHDERFAhligqrQLAEKYYRVSKDEN
           . LD .N. S .A .::    R: .:N Q.IVI:  ....      : A:... V ..::
           AALDeaNV.sRvAnyIk....rerskna QFIVIsLRnnmm......ekADaLvGVymqdd

           QHSIIESQEI    1314
             S : S ::
           gvskVislkL     210
```

Legend of Alignment

: positive score

. score between -2 and 0

Please report problems and feedback concerning bioSCOUT through the support interface.

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Alignment: 1116E.rad50.sid2 - pfam|hmm|sigma70_ner

HMMPFAM - alignment of 1116E.rad50.sid2 against pfam|hmm|sigma70_ner

Sigma-70, non-essential region -

- This hit is scoring at : -122.2
- Scoring matrix : BLOSUM62 (used to infer consensus pattern)

```
Q:    112 ---AIESVLQTINPHTGEKVCLSYR---CADMDREIPALMG---SVSKAVLENVIFV---
              .:: :L...:  . E:  LS       .D D  I..  .       :: L..
H:      1 fPgtvdyILaeYdRvetEegRLsDilsGyiDPddgiapdeAPtathieselaeepssekd

          ------------------HQDESN----WPlqDPSTLKKKFDDIfsATRYTKALEVIK--
                             ..D:S.     P  DP..:::F.::  ..:..K. :.:K
          daadaddddDEdEeeeessdddsEagdggp..DPEeArerFgel..reqlektkkalkKh

          ----KLHKDQMQEI----KTFRLKL---ENL-QTVKDQAHKLREN--------------
              K   :.::.:    ...:L     : L :.V:....::R:.
          GRgskqalealeaLAelFmpikLvPKQfDaLVervRgmldrvRkqERaIMklCVrdArMP

          ----IAQ-----------DQEKSDASKS--QMEQLKEKICGTEREILQME     260
              I..            . KS... :  .:E::KE.I. .:::::..:E
          RkdFiksFpgnETnleWlekllkskkkyadeaLervkedIlrcQqKLadlE     227
```

Legend of Alignment

: positive score

. score between -2 and 0

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Alignment: 1116E.rad50.sid2 - pfam|hmm|DUF259

HMMPFAM - alignment of 1116E.rad50.sid2 against pfam|hmm|DUF259

Protein of unknown function, DUF259 -

- This hit is scoring at : -137.1
- Scoring matrix : BLOSUM62 (used to infer consensus pattern)

```
Q:     441 DGQIQSKIESMSGILRRRKDKEKERDAAEVELSK----------*-----FNLSRID---
            ..:::.::.:.G  ...:: ::.R...:V:...                F  :::D
H:       1 adamRamLDqLMGRdasnRngdesrqkkkvkfdDpeVCrsyLvgfCPHDlFinTKmDnLG

          ------ERERHMQIEVERKTLALG---ERDYDSIISQ------KRTEVYSLEQKIKVLLR
                : : ....E ..K:  .    ERD  ..:.:      :..E:..L.:KIK  ::
          pCpkvHdlklradYErasksrdyfpkfErdaleflersvsEvtqsPEiLELsEkikEkmk

          EKDIinrnADERVKLGLKKDALESSKDKLNEIVNEHK--DKIKKVLRGR----------
          E. :     D   ::. .K..LE.:::: .: . E.K   :  . L  .
          EAfv....hDcdrridkakqrLeetqeeqtkeaaeekRqaeelaeldeekAsLPqPvPAq

          -----------------NPFEKDM-----KKEINQAFWPVdKEYNELRSKSQEAEQ---E
                           ... .:.     : ::::A   : KE..EL::K.:E.E:   E
          PPssELPPPDPRtqEvIgkllaeaEaLGeeGkVdeaqklm.kevEeLkakkkeleeklde

          LKFTQSKVTDAreqltklrrdmdakrrfldsklqsilqisanvdmfpkVLQDAMNKR---
          :: .... ..A                                          D.M.::
          vrnaapssaqa.....................................WslDeMgqqKLr

          ------------DEQKRLENFANG--------MREMLAPFEHLARK-----NHVCPCCER
                     D..:RL.:. .G         :RE.LA..:...K     ...    ER
          VCEvCGAyLsvlDadrRlADHfgGKlHLGYvkiReklaeLkkakaklkeevkktgrekeR

          AFTPDEEDEFVKKQRMQ     717
          . .. E.:: :. QR..
          eereretektldgqRqh     335
```

Legend of Alignment

: positive score

. score between -2 and 0

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Alignment: 1116E.rad50.sid2 - pfam|hmm|filament

HMMPFAM - alignment of 1116E.rad50.sid2 against pfam|hmm|filament

Intermediate filament protein -

- This hit is scoring at : -203.9
- Scoring matrix : BLOSUM62 (used to infer consensus pattern)

```
Q:     840 ---EEIQlELN-----------FLQRTRDTLIVEVDDLRDQH--k---R-MLNEDMSSAQ
               E::Q .LN           FL:::...L V::::LR.:.      . .   .: ..:
H:       1 nEKeqmQ.nLNDRLAsYIdKVRfLEqqNkeLevkieelrqkqsrggpasvsrlyslYete

            VRwhNAREEKVKASSILERFQKSEEELVLLAEE-KEQLIV-------------EKKL--L
            :.  ..R.:  :.::  .R.Q . :.L  ..E: :::.                E .L
            ie..eLRrqidqltnerarlqlEidnlrealedfrkKyedKeDLaaQnqlkdlEialntk

            EESLDPLSkEKESLLQEYNAL------------KQKLDEEY--------------HQLAE
            E..L ..  E:.. .:. .L           . ::.LDE.                .:LA
            eaeLaTaL.eRqeaEndlvgLRaQiAklEslaaRkdlDeaTLarvDLEnkvEsLqEElaF

            RKREFQQELDAL-----GR--LNMKIKGYL----DSKKNEKLKELQgrhvlchSQLQsCM
             K:..::E:.L     ..  :N::::..      . . .:.L:E::       :Q.:  :
            LKknHeEEvkeLqaqiqdtgqvnVEmDaarqqEwklDLtkaLrEiR.......aQYE.ei

            AKQQRISAELNKSKELLQGQGQLKRNIDDnlkYRKTKADVEQLTRDIESLEERLLSIgsL
            A::.R .AE   ..:L : Q ...RN :    .R..K.::.:L.R.T:SLE .L S:
            AeknrqeaEewYksKleeLqLaaaarngea...lrsaKeEitElRRqiQsLeiELqsl..K

            SAIEADLKRHSQEKERLNSEFNRWQGTLSVYQSNISKHKQELK--LSQYKDI---------.
            S. .:  :: :: :ER..:E. ::Q..:S .:..:.: ::E:.  L.:Y:::
            sqnasLErqlaElEeryeaelaqyqalisqlEeeLqqlreEMarqLrEYQeLLdVKlaLD

            -------------EKR    1118
                        E.R
            iEIATYRKLLEGEEsR     359
```

Legend of Alignment

: positive score

. score between -2 and 0

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bioSCOUT®

Projects | Analyses | Admin | Alert | SRS | Tutorial | Help | Features

Alignment: 1116E.rad50.sid2 - pfam|hmm|Myosin_tail

HMMPFAM - alignment of 1116E.rad50.sid2 against pfam|hmm|Myosin_tail

Myosin tail -

- This hit is scoring at : -555.1
- Scoring matrix : BLOSUM62 (used to infer consensus pattern)

```
Q:     237 ASKSQMEQLKEKICGTEREILQMETSLDE----------LRRLQGQI----DIKATERS
             :.Q..:L:E::  .E.E: Q:...L::           ::.L:.:I    :  ..ER:
H:       1 dlerqkreleeqlkrkeselsqlslklEdEqalvaqLqkkikeleaRIeELeEeLEaERa

          T------LLTQQHEKLAALSEENEDT-------------DEELM-------EWQTKFEE
          .        .. ..:L..LSE. E:.              :.EL.       E ..:.EE
          ARaKaEkqRaDLsrELEeLsERLeEagGaTaaQiElnkKREaELaKLRrdLEEanlqhEe

          RIALLETKISKLVRDMDDE----ASYSSVLSKQNSELTHEIGKLQAEADAHLTMKHERDS
          .:A.L..K ...:.::.D:    ....: ..K:.S:L. E:..L A:.D: ...K ..:.
          alatLRKKHqdainElsdQieqLqKqKakaEKeKsqlqaEvddllaqldsitKaKlnaEK

          DIKNICTKHNLGPVPehpfTNDVAMNLT--NRIKARLSSLENDLLDKKKSNEDQLDVLWK
          ..K.: :: :  .V.    .:::...L.  .. K:RL.S .:DL. : :. E Q:. L K
          kakqlEsQlsElqvK....ldElqRqlnDltsqKsRLqsENsdLtrqleEaEaqvsqlsk

          HYLKINA------RYSEVDGQIQSKIESMSGILRRRKDK-----EKERDA---AEVELSK
             ::.:      R  E :.: ::.:::.   L..  D.     E:E.:A   .E :LSK
          lKsqlesQLEeAkRslEEEsReRanLqaqlrnlehDlDslrEqlEEEsEAKaeleRqLsK

          FNlSRIDERERHMQIEVERKTLALGE--RDYDSIISQKRTEVYSLEQKIKVLLREKDIIN
           N :.I.: ....: E . :. .L E  :..:. IS: .... :...K.. L :.K. :.
          an.aeiqqwrsKfEsEgalraEElEElKkKlnqkisElEeaaEaanaKcssLEKtKsRLq

          RNAD----ERVKLGLKKDALESSKDKLNEIVNEHKDKIKKVLRGRNPFEKDMKKEINQAF
          ...:    E  :.......LE..:...::I: E K.K:.:: .  .. :::.:.  .:.F
          sElEDlqievEranaaaseLEKKqknFDKilaEwKkkvdelqaEletAqreaRnlstElf

          WpVDKEYNELRSKSQEAEQELKFTQSKVTDAREQLTKLRR---DMDAKRRFLDSKLQSIL
            :..E..EL:.: :...:E K .Q.::.D..:QL :  R   ::::..RR L::: ..:
          r.lKneleElkDqvEaLrRENKnLqdEikDLtdqLgEgGRnvHELEKarRrLEaEkdELq

          QisANVDmfpkvLQDAMnKRDEQKRLENFANgMREMLAPFE-HLARKnhvcpcceraftp
          .  A  :      .: A: :::E.K L.  .. :.:: :..E .LA.K
          a..ALeE.....AEaAL.eqeEsKvlRaqvE.lsqiRsEiERRLaEK.............

          DEEDEFVKKQRMQnsstaerskalAMESSNAEaLFQQLDKLRTIYDAYVKLVEETIP---
          :EE E .:K...:           A:ES .A. L :.  K :.   .. K :E .I
          EEEfEntRKnhqr...........aiesLqas.LaEaEaKgKaEalRlKKKLEgdInELE

          LAEKNLNQHLADESQKAQAFDDLL-GVLAHVQMDRDAVEALLQPTDTIDRHVHEIQQlvk
          :A .:.N:  A:..:.:.:.. :  : ..V: :: A E . :.. ..:R.. .:Q.
```

```
iaLDhaNkanaeaqKnvKkyqqqvkeLQtqvEeeQRaredareqlavaERRataLqa...

EVEDLEYALDSSGRGVKSLEeiqLELNFLQRTRDTLIVEVDDLRDQHRMLNEDMSSAQVR
E:E:L..AL:.: R. K..E   .EL  .... :.L..: ..L  Q.R.L. :::::.Q
ElEELrvaLeqaeRaRKqAE...tElaEaservneLtaqnssLiaqKRKLEgelaalqsD

WHNAREEKVKAssiLERFQKSEEELVLLAEEkeqLIVEKKLLeESLDPLSKEKESLLQEy
 ..A .E  .A    ER :K:.:.. LAEE   L  E::   :.L: L.K: ES ::E
LDEavnElkaA...eERakkaqaDaarLaeE...LrqEQehs.qklErlRKqLEsqvKe.

naLKQKLDE-EYHQLAE----------RKREFQQELDALGRLNMKIKGYLdSKKNEKLKE
  L: :LDE E .L.           R RE.:.ELD.  R :.:.:  L .K...::KE
..LqvRLdEaEaaAlkgGKkvIqKLEaRVReLEaELdgEqRRhaetqKnl.RKaeRrvKE

LQG------RHVLchsQLQSCMAKQQrisaelNKSKellqgqgQLKRNIDDNLKYRKTK-
LQ       :::    :LQ..: K Q       K K       ..KR.:::  :..:..
LqfQvEEDkKnle...rlQDLvDKLq......aKiK.......tyKRQlEEaEEiaqinl

ADVEQLTRDIESLEERL-LSIGSLSAIE---ADLKRH    1075
:...:..R::E..EER.  : .SL:.:.   A. :R.
sKyRkaQreLEdAEERADqAEsslnklRqreaKsRrs     864
```

Legend of Alignment

: positive score

. score between -2 and 0

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SeqWeb Sequence Analysis

HmmerPfam Results

Refine

Query: 1116SID2 from: 1 to: 1316 WPDEF Case 1116 Rad50 SEQ ID NO: 2

Scores for sequence family classification (score includes all domains):

Model	Description	Score	E-value	N
Rad50_zn_hook	Rad50 zinc hook motif	36.3	6.9e-07	2
HSP9_HSP12	Heat shock protein 9 / 12	-11.4	4.1	1
LEA_1	Late embryogenesis abundant (LEA) group	-14.3	5.6	1
Histone_HNS	H-NS histone family	-28.3	7.9	1
ABC_tran	ABC transporter	-46.1	0.14	1
SMC_N	RecF/RecN/SMC N terminal domain	-69.5	0.75	1
TACC	Transforming acidic coiled-coil-contain	-	88.9	8 1
DUF164	Uncharacterized ACR, COG1579	-90.1	4.1	1
Tropomyosin	Tropomyosin	-	91.4	8 1
SMC_C	SMC family, C-terminal domain	-116.7	0.36	1
sigma70_ner	Sigma-70, non-essential region	-122.2	8.3	1
filament	Intermediate filament protein	-203.8	3.3	1

SeqWeb Sequence Analysis

ERM Ezrin/radixin/moesin family -236.2
9.5 1

Parsed for domains:

Model	Domain	seq-f	seq-t		hmm-f	hmm-t		score	E-value
Rad50_zn_hook	2/2	673	726	..	1	57	[]	32.5	9.9e-06

Alignments of top-scoring domains:
Rad50_zn_hook: domain 2 of 2, from 673 to 726: score 32.5, E = 9.9e-06

```
                  *->galesekaelkkaieeleeeessccCPvCgReLgteeekkelikeyks
                     +  ++++++l ++   +++ +   CP+C+R + t++e+ e++k+++
    1116SID2   673    NFANGMREMLAPFEHLARKNHV--CPCCERAF-TPDEEDEFVKKQRM
716

                  eldrlpeelk<-*
                  + +++ e  k
    1116SID2   717 QNSSTAERSK    726
```

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Protein families database of alignments and HMMs

Wellcome Trust Sanger Institute

Rad50_zn_hook

Figure 1: 118d Replication
Rad50 coiled-coil zn hook

Key:

Domain	Chain	Start Residue	End Residue
Rad50_zn_hook	A	421	475
Rad50_zn_hook	B	421	475

The Swissprot/PDB mapping was provided by MSD

Accession number: PF04423

Rad50 zinc hook motif

Add Annotation

The Mre11 complex (Mre11 Rad50 Nbs1) is central to chromosomal maintenance and functions in homologous recombination, telomere maintenance and sister chromatid association. The Rad50 coiled-coil region contains a dimer interface at the apex of the coiled coils in which pairs of conserved Cys-X-X-Cys motifs form interlocking hooks that bind one Zn ion. This alignment includes the zinc hook motif and a short stretch of coiled-coil on either side.

INTERPRO description (entry IPR007517)

The Mre11 complex (Mre11 Rad50 Nbs1) is central to chromosomal maintenance and functions in homologous recombination, telomere maintenance and sister chromatid association. The Rad50 coiled-coil region contains a dimer interface at the apex of the coiled coils in which pairs of conserved Cys-X-X-Cys motifs form interlocking hooks that bind one Zn ion. This alignment includes the zinc hook motif and a short stretch of coiled-coil on either side.

Alignment	Domain organisation

◉ Seed (17) ○ Full (25) Format: Coloured alignment Get alignment View HMM logo Further alignment options here Help relating to Pfam alignments here	◉ Seed (17) ○ Full (25) As a Graphic | As a Tree Zoom 0.5 pixels/aa. ☐ Bootstrap tree View Graphic NIFAS Applet To find out about the NIFAS tree-viewer, click here
Species Distribution	**Phylogenetic tree**
NEW! View alignments & domain organisation by species Tree depth : Show all levels View Species Tree	◉ Seed (17) ○ Full (25) Download tree ATV Applet The trees were generated using Quicktree To find out more about ATV phylogenetic tree-viewer click here

Database References	
PDB You can find out how to set up Rasmol here	1l8d A; 421; 475; PDB-2-Pfam Scop|Cath|Pfam Rasmol (unix) Chime (pc) CATH-PDBSUM SCOP-UK SCOP-USA MSD
SYSTERS	Rad50_zn_hook
PANDIT	Rad50_zn_hook

Literature References

1.
Tethering on the brink: the evolutionarily conserved Mre11-Rad50 complex.
Connelly JC, Leach DR;
Trends Biochem Sci 2002;27:410-418.

2.
The Rad50 zinc-hook is a structure joining Mre11 complexes in DNA recombination and repair.
Hopfner KP, Craig L, Moncalian G, Zinkel RA, Usui T, Owen BA, Karcher A, Henderson B, Bodmer JL, McMurray CT, Carney JP, Petrini JH, Tainer JA;
Nature 2002;418:562-566.

Pfam specific information

Author of entry	Bateman A
Type definition	Motif
Alignment method of seed	Clustalw
Source of seed members	Bateman A
Average Length	55.6
Average %id	24
Average Coverage	5.34%

HMMER build information

	Pfam_ls [Download HMM]	Pfam_fs [Download HMM]
Gathering cutoff	25.0 25.0;	25.0 25.0
Trusted cutoff	40.9 40.9;	31.4 38.9
Noise cutoff	22.6 22.6;	24.6 20.6
Build method of HMM	hmmbuild -F HMM_ls SEED hmmcalibrate --seed 0 HMM_ls	hmmbuild -f -F HMM_fs SEED hmmcalibrate --seed 0 HMM_fs

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Protein families database of alignments and HMMs

Wellcome Trust Sanger Institute

Mre11_DNA_bind

Accession number: PF04152

Previous identifiers: Mer11_DNA_bind;

Mre11 DNA-binding presumed domain

The Mre11 complex is a multi-subunit nuclease that is composed of Mre11, Rad50 and Nbs1/Xrs2, and is involved in checkpoint signalling and DNA replication [1]. Mre11 has an intrinsic DNA-binding activity that is stimulated by Rad50 on its own or in combination with Nbs1 [2].

INTERPRO description (entry IPR007281)

The Mre11 complex is a multi-subunit nuclease that is composed of Mre11, Rad50 and Nbs1/Xrs2, and is involved in checkpoint signalling and DNA replication [MEDLINE:21984524]. Mre11 has an intrinsic DNA-binding activity that is stimulated by Rad50 on its own or in combination with Nbs1 [MEDLINE:20300914].

Alignment

Seed (10) Full (20)

Format: Coloured alignment

Get alignment

View HMM logo

Further alignment options here

Help relating to Pfam alignments here

Domain organisation

Seed (10) Full (20)

As a Graphic | As a Tree

Zoom 0.5 pixels/aa. Bootstrap tree

View Graphic NIFAS Applet

To find out about the NIFAS tree-viewer, click here

Species Distribution

NEW! View alignments & domain organisation by species

Tree depth: Show all levels

View Species Tree

Phylogenetic tree

Seed (10) Full (20)

Download tree ATV Applet

The trees were generated using Quicktree

To find out more about ATV phylogenetic tree-viewer click here

Database References

SYSTERS	Mre11_DNA_bind

PANDIT	Mre11_DNA_bind

Literature References
1. A mechanistic basis for Mre11-directed DNA joining at microhomologies. Paull TT, Gellert M; Proc Natl Acad Sci U S A 2000;97:6409-6414.
2. The Mre11 complex: at the crossroads of dna repair and checkpoint signalling. D'Amours D, Jackson SP; Nat Rev Mol Cell Biol 2002;3:317-327.

Pfam specific information	
Author of entry	Wood V, Finn RD
Type definition	Domain
Alignment method of seed	Clustalw
Source of seed members	Pfam-B_3909 (release 7.3);
Average Length	200.3
Average %id	39
Average Coverage	28.59%

HMMER build information		
	Pfam_ls [Download HMM]	Pfam_fs [Download HMM]
Gathering cutoff	25.0 25.0;	25.0 25.0
Trusted cutoff	71.9 71.9;	49.5 32.0
Noise cutoff	13.1 13.1;	8.2 17.4
Build method of HMM	hmmbuild -F HMM_ls SEED hmmcalibrate —seed 0 HMM_ls	hmmbuild -f -F HMM_fs SEED hmmcalibrate --seed 0 HMM_fs

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